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STUDIES IN INDIAN TOBACCOS.

4. PARTHENOCARPY AND PARTHENOGENESIS IN TWO VARIETIES OF NICOTIANA TABACUM L.—VAR. CUBA AND VAR. MIRODATO.

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I. INTRODUCTION.

THE occurrence of parthenogenesis in the genus *Nicotiana* has been under investigation for some years in various parts of the world. The subject was first brought forward in 1909 by Mrs. R. Haig Thomas[1] in a paper entitled *Parthenogenesis in Nicotiana*. In this paper, a series of experiments was described in which castration of the flowers of various species and varieties of *Nicotiana* was followed, almost invariably, by the production of viable seed. In some experiments the anthers were removed, in others both stigmas and anthers; in nearly all cases seed resulted. All the usual precautions—sterilization of the instruments and protection of the buds by means of paper bags—were employed. Among the varieties tested were *Nicotiana Tabacum* var. *Cuba* and *Nicotiana Tabacum* var. *Mirodato*. The seed of the former was gathered from a plant in the garden of Casa Loring near Malaga said to

[1] Haig Thomas, R., *The Mendel Journal*, I. 1909, p. 5

have been grown from seed imported from Cuba. The seed of *N. Tabacum* var. *Mirodato* was obtained from the Board of Trade and came originally from Asia Minor.

The following results were obtained with these two varieties and their hybrids :—

Variety	Number of successful experiments	Notes
Nicotiana Tabacum Cuba ..	5 sprays in 2 plants	Parthenogenetic seed sowed 9th September, germinated on the 21st September
Nicotiana Tabacum Mirodato	1 spray on 1 plant	Asia Minor, seed obtained from the Board of Trade
F_1 Nicotiana Sylvestris × Nicotiana Tabacum Cuba	2 sprays on 2 plants	
Nicotiana Tabacum Cuba × F_1 Purple (Sylvestris × F_1 Red Sanderæ × Affinis)	3 sprays on 3 plants	

In the case of *N. Tabacum* var. *Cuba* half the parthenogenetic seed was round, full and sound looking, the other half flattened, poor and little likely to germinate.

The author sums up her results as follows : " The fact remains that parthenogenesis was discovered in ten species, varieties and hybrids of *Nicotiana* and it is possible will be found in all of them if the right period is chosen for the trial, i.e., when the plant is beginning to go off its fullest blossom . . . . In the Tabaccums success was unfailing." Further confirmatory evidence is given by Mrs. Haig Thomas in a letter quoted by Goodspeed[1] in 1915, " Since then (the appearance of her original paper in the *Mendel Journal*) I have every season produced parthenogenetic seed from one or more *Nicotiana* though the conditions for growing the plants here (Moyles Court, Ringwood) are not so favourable as at Creech (?) Grange, Wareham, where I first made trials for parthenogenesis and succeeded in so many varieties."

It is obvious that parthenogenesis, to the extent described in the above paper, would introduce serious errors into any experiments on tobacco breeding. Investigations were, therefore, started at several tobacco breeding centres to ascertain how far the formation of parthenogenetic seed was characteristic of other species and varieties of *Nicotiana*. In 1910 and 1911, a detailed

[1] Goodspeed, T. H., *University of California Publications in Botany*, Vol. V, 1915, p. 249.

investigation[1] was carried out by one of us on fifty one Indian types[2] of *N Tabacum*. In each type, 50 to 100 buds were castrated on each of two plants. In some, the anthers were removed, in others both the anthers and stigmas. Plants were chosen at all periods of flowering. In 1911, the experiment was repeated on six types (9, 51, 16, 35, 23, 38). In the thousands of flowers castrated only five seed-bearing capsules were found. These occurred on three plants. As the percentage is so small (5 in 10,000), there is always the possibility that these might be due to accidental errors of technique. In addition, in nine first generations, raised in Pusa during 1908 to 1913 (each culture containing about one hundred plants), no individuals resembling the mother plant have ever been detected. With the Indian types grown at Pusa, therefore, practically no evidence of parthenogenesis was obtained.

Several investigations on this subject have been carried out in America. In 1913, Wellington[3] published an account of a very thorough study of this matter. Attempts were made to induce parthenogenesis in various species and varieties of *Nicotiana* by (1) the removal of anthers or of anthers and stigmas, (2) mutilations such as singeing or tickling the buds, (3) exposure to various vapours such as chloroform and ether and (4) injection of certain liquids. No fruits or fertile seed were produced by any of these methods with the exception of one capsule among ninety-eight emasculated blossoms on a plant of *N. plumbaginifolia*. This is ascribed to an error in technique. In a few cases, however, abortive seeds were produced by mutilation and artificial stimulation. As regards the castration and decapitation experiments, in over 1,000 buds treated, no fertile or abortive seed was produced (with the exception of the above-mentioned single capsule). Hayes and Beinhart[4] in a communication issued in 1914 make the following statement: "We have made numerous attempts to secure parthenogenetic seeds from various species of *Nicotiana* without success."

The following investigations have been carried out in Europe. In connection with a paper read by Mrs. Haig Thomas[5] at the Paris Conference of Genetics, Bateson[6] stated that he had been unable to obtain parthenogenetic

[1] Howard, G. L. C., *Mem. of the Dept. of Agr. in India*, Botanical Series, Vol. VI, 1913, p. 25.

[2] Howard, A., and Howard, G. L. C., *Mem. of the Dept. of Agr. in India*, Botanical Series, Vol. III, 1910, p. 59.

[3] Wellington, R., *Amer. Natur.*, Vol. 47, 1913, p. 279.

[4] Hayes, H. K., and Beinhart, E. G., *Science*, Vol. 39, 1914, p. 284.

[5] Haig Thomas, R., *4th Cong. Int. Gen. Rep.* 1913.

[6] Bateson, W., 4th *Cong. Int. Gen. Rep.* 1913.

seed except in the case of *N. Tabacum* var. *Cuba*. Fruwirth[1] also made a series of experiments with a pure line of *N. Tabacum*. No parthenogenetic capsules were obtained although various modes of procedure were adopted. The corolla remained fresh longer than usual and the ovary swelled a little but sections through this organ showed that it was filled with parenchymatous tissue and that no seeds were present. Fruwirth concludes therefore that the tendency towards parthenocarpy is present to a small degree; fruit formation begins but the seedless fruits are thrown off before they attain their full size. In none of these investigations, except those of Bateson, had the varieties used by Mrs. Haig Thomas been employed.

In 1915, Goodspeed[2] published a paper entitled *Parthenogenesis, parthenocarpy* and *phenospermy in Nicotiana* in which he described very detailed and elaborate experiments on plants of *Nicotiana Tabacum* var. *Cuba* obtained from seed supplied by Mrs. Haig Thomas. At the same time, efforts were made to produce parthenogenetic seed in a large number of other species and varieties of *Nicotiana* but without success. Over 1,500 attempts yielded entirely negative results. The experiments with *N. Tabacum* var. *Cuba* were carried out with the greatest care and every precaution to eliminate error was adopted. About 800 buds, divided approximately evenly between ninety-five plants, were treated by one of the following methods (1) removal of the anthers, (2) removal of the anthers and stigma. (3) removal of the stigma. It was found that, in general, the flowers and maturing capsules, though they may have fallen ultimately, remained attached to the plant for a much longer period of time than in the case of other species and varieties of *Nicotiana* when similarly treated. Moreover, out of the 800 castrations and mutilations, over 100 normally matured capsules were produced. In the majority of these parthenocarpic fruits, empty seeds were found in great numbers. In nine of these capsules, however, approximately 50 seeds were found, some of which showed normally matured endosperm and embryos. Some of the seeds germinated and produced normal seedlings. Eighteen seeds gave six seedlings. Goodspeed was therefore able to show that the behaviour of *N. Tabacum* var. *Cuba* in producing parthenocarpic fruits on castration was very different to that of the majority of the species and varieties of *Nicotiana*. He was also able to produce a *small* quantity of seed which, considering the precautions and care taken, must be assumed to be of parthenogenetic origin. The very small amount of seed so produced forms, however, a great contrast to the ease with which such seed was produced on the same variety in England.

[1] Fruwirth, C., *Zeitschrift für Pflanzenzüchtung*, Bd. II, 1914, p. 95.
[2] Goodspeed, T. H., *University of California Publications in Botany*, Vol. V, 1913, p. 249.

Goodspeed calls attention to the *possibility* that after one or two years of further cultivation the large proportion of phenospermic seeds, with or without embryos, may be lessened in favour of a greater proportion of entirely normal, viable seeds resulting from the castration or mutilation of flowers of *Nicotiana Tabacum Cuba*. He suggests that differences in soil and climate may account for the discrepancy between the results with this variety in England and in California.

The power possessed by *N. Tabacum* var. *Cuba* of holding the capsules after castration and producing phenospermic seed is apparently a dominant character for Goodspeed and Ayres[1] found that this quality was exhibited by the F_1 hybrids with *N. sylvestris*. The plants of the F_1 generation (as in other species hybrids with *N. sylvestris*) produced no functional pollen and no viable seed under bag. Mature normal fruits with phenospermic seed were, however, obtained.

The present investigation originated from a request, made by Mrs. Haig Thomas in 1920 to one of the authors, that experiments should be made to determine whether the contrast between the negative results obtained at Pusa in 1910 and those obtained by her was due to the varieties used. As it seemed desirable to ascertain whether the types which gave such abundant parthenogenetic seed in England would do so under Indian conditions, arrangements were made to carry out the investigation. Three packets of seed were received from Mrs. Haig Thomas in 1920 which were described as follows :—

(1) *Nic. Tab. Cuba*, 1917 seed ; Casa Loring Garden, Malaga ; foliage scentless.

(2) *Nic. Tab. Mirodato*, scented foliage ; Asia Minor—imported by the Board of Trade and grown for ten years by R. Haig Thomas.

(3) Seed gathered from F_2 *Nic. Tab. Cuba* (ovule parent) × *Nic. Tab. Mirodato* (pollen parent).

The experiments were begun in October 1920 and have been continued during the seasons 1921-22 and 1922-23. The seeds were sown in boxes in the manner described in a former paper[2] with all the precautions normally used at Pusa in raising cultures for investigations on tobacco breeding. No difficulty has been experienced in germinating the seed or in growing these varieties at Pusa. Although slightly slower in growth than the indigenous kinds, they have given quite as good plants. Good seed was obtained both under bag and from free-flowering plants. The flowers were treated by the removal

[1] Goodspeed, T. H. and Ayres, A. H., *University of California Publications in Botany*, Vol. V, 1916, p. 273.

[2] Howard, G. L. C., *loc. cit.*

of the anthers or of both anthers and stigma. Full precautions to prevent contamination were taken. The instruments employed were sterilised and the buds were protected by means of parchment bags. Plants in all stages of flowering were used. The results in the main agree with those of Goodspeed but it has not been possible to produce parthenogenetic seed in *N. Tabacum* var. *Cuba*. In considering the experiments on the two varieties and the hybrid, it will be best to deal with each kind separately.

II. Experiments with N. Tabacum var. Mirodato.

Four cultures of this variety were raised. In each of the years during which the investigation was in progress, a culture was raised from the original seed; in 1922-23 one culture was grown from seed harvested in India in 1921.

Table I.

Results with Nicotiana Tabacum var. Mirodato.

Season	Origin of culture	Nature of operation	Date	No. of plants	No. of capsules per plant treated	No. of capsules which developed
1920-21	Original seed	Stamens and stigmas removed	8-3-21 to 14-3-21		25	None
Large well developed plants					30	,,
					30	,,
			Early stage of flowering	7	30	,,
					30	,,
					30	,,
					32	,,
		Do.	9-3-21 to 15-3-21		36	None
					31	,,
					46	,,
			Early stage of flowering	4	39	1 capsule but bag torn
		Do.	23-3-21 Flowering just going over	2	15	None
					11	,,
		Do.	25-3-21 Flowering very nearly over	2	21	None
					13	,,
		Stamens removed	8-3-21 to 15-3-21	..	32	None
					30	,,
					30	,,
			Early stage of flowering		32	,,
					38	,,
					31	,,

TABLE I—(*contd.*)

Season	Origin of culture	Nature of operation	Date	No. of plants	No. of capsules per plant treated	No. of capsules which developed
1920–21	Original seed —*contd.*	Stamens removed	9–3–21 to 15–3–21 Early stage of flowering	4	27 37 35 34	None " " "
LARGE WELL DEVELOPED PLANTS —*contd.*		Do.	23–3–21 Flowering just going over	2	9 20	None "
		Do.	25–3–21 Flowering very nearly over	2	13 19	None "
1921–22	Original seed	Stamens and stigmas removed	25–3–22	2	37 39	None "
PLANTS NORMALLY DEVELOPED		Do.	1–3–22	1	56	"
		Stamens removed	25–2–22	1	44	3 appeared to set but did not develop and fell early
		Do.	1–3–22	2	57 50	39 Ditto 18 Ditto
1922–23	Original seed	Stamens and stigmas removed	12–3–23 and 18–3–23	2	9 9	None "
PLANTS SMALL OWING TO LATE PLANTING		Stamens removed	12–3–23 and 18–3–23	2	15 9	None "
	Seed from 1921 harvest	Stamens and stigmas removed	12–3–23 and 18–3–23	2	13 11	None "
		Stamens removed	12–3–23 and 18–3–23	2	13 17	None "

It will be seen that in 1921, 806 buds (distributed on 29 plants) were treated but no normal capsules were formed. A single capsule was found on one plant but as the bag had been accidentally torn this must be disregarded. The capsules after castration were not held by the plant but were shed shortly after the operation. No seed was formed. In 1922, 283 buds (distributed on 6 plants) were treated with similar results. Some of the fruits began to swell slightly but these dropped off before they attained any size. Similarly in 1923, 96 buds (on 8 plants) gave no capsules. Thus in the three years 1921 to 1923, 1,185 buds gave only one capsule (which was obviously due to a torn bag). *Nicotiana Tabacum* var. *Mirodato*, therefore, under Indian conditions shows neither parthenogenesis nor parthenocarpy. This can scarcely be attributed to any adverse effect of the climate on the variety as good seed was obtained both under bag and from free-flowering plants.

III. Experiments with Nicotiana Tabacum var. Cuba.

The results obtained with *Nicotiana Tabacum* var. *Cuba* are given in Table II. As in the case of *N. Tabacum* var. *Mirodato*, cultures were raised from the original seed every year. In 1922-23, an additional culture was grown from seed harvested in India in 1921.

Table II.

Results with Nicotiana Tabacum var. *Cuba.*

Season	Origin of culture	Nature of operation	Date	No. of plants	No. of capsules treated per plant	No. of capsules which developed	No. of seeds which germinated
1920–21	Original seed	Stamens and stigmas removed	13–4–21 to 18–4–21	6	23	5	None
Large well developed plants					16	11	"
					16	8	"
					17	6	"
					?	10	"
					?	5	"
		Stamens removed	13–4–21 to 18–4–21	6	38	26	None
					24	18	"
					33	29	"
					17	15	"
					?	8	"
					?	7	"

TABLE II—(*contd.*)

Season	Origin of culture	Nature of operation	Date	No. of plants	No. of capsules treated per plant	No. of capsules which developed	No. of seeds which germinated
1921–22 PLANTS NORMALLY DEVELOPED	Original seed —*contd.*	Stamens and stigmas removed	24-3-22	2	31	5	None
					34	10	,,
		Do.	25-3-22	1	38	9	,,
		Do.	2-4-22	1	23	17	,,
		Stamens removed	22-3-22	2	30	21	None
					37	21	,,
		Do.	23-3-22	1	48	17	,,
		Do.	2-4-22	1	26	19	,,
1922–23 PLANTS SMALL OWING TO LATE PLANTING	Original seed	Stamens and stigmas removed	1-4-23	1	8	None	
		Stamens removed	1-4-23	1	6	None	
	Seed from 1921 harvest	Stamens and stigmas removed	20-3-23	1	13	None	
		Stamens removed	24-3-23	1	20	None	

It will be seen that the results obtained with this variety are very different from those with var. *Mirodato*. In 1921, one hundred and eighty-four buds (distributed on 8 plants) gave no less than 118 capsules. Moreover, some capsules were formed on all the plants treated. These capsules remained attached to the plant but were not quite so large as the normal fruits. They

were filled with structures which looked like seeds but which were somewhat smaller and much lighter both in weight and colour. They were also easily crushed between the fingers. Examination showed that the seed coats were empty. No seeds with embryos were found in the portion tested. The remainder was sown in boxes in October 1921 with the greatest care. Not a single seed germinated. At the same time, some of the original seed was sown and germinated well. In 1922, 119 similar capsules were obtained from 267 treated buds (on 8 plants) and again every plant produced capsules. The seeds were of the same nature as in the previous year. Examination under the microscope, sowing in boxes in earth, and germination in Petrie dishes showed no fertile seed. No capsules were obtained in 1923 from 47 buds (on 4 plants). At Pusa, therefore, it has not been possible to obtain any parthenogenetic seed from *N. Tabacum* var. *Cuba*. The plants, however, exhibited marked parthenocarpy.

An examination of the percentage of parthenocarpic fruits brings out one interesting point, namely, an apparent connection between the vigour of the plant and the number of capsules formed. In 1920-21 when the plants were exceptionally large and vigorous, the percentage of fruits was 64. Average sized plants in 1921-22 gave a percentage of 44. In 1922-23, owing to adverse climatic conditions, the seedlings could not be transplanted until late in the season. The plants remained poor and small in consequence. In this year no parthenocarpic fruits at all were obtained.

IV. Plants from the cross N. Tabacum var. Cuba × N. Tabacum var. Mirodato.

In the case of the hybrid, cultures were grown from the original seed in 1920-21 and in 1921-22. In 1922-23 two cultures were raised from seed harvested in India in 1921 and in 1922. In both cases these seed plants had been grown from Mrs. Haig Thomas' original seed. In addition, in 1921-22 a culture was raised from some apparently parthenogenetic seed obtained in 1921. Seed was harvested from certain plants of this culture in May 1922 and sown in October 1922.

The results obtained with the hybrid are a little more difficult to describe on account of the variability of the material. No statistical examination of the morphological differences was made but the colour of the corolla of the plants operated on was recorded. Three different colours occurred—white,

pink and white with splashes of pink (termed parti-coloured in the Table). The detailed results are given in Table III :—

TABLE III.

Results with the progeny of the cross N. Tabacum Cuba ♀ × N. Tabacum Mirodato ♂—seed collected by Mrs. Haig Thomas from F_2 generation.

Season	Origin of culture	Nature of operation	Date	No. of plants and colour of corolla	No. of capsules per plant treated	No. of capsules which developed	No. of seeds which germinated
1920–21	Original seed	Stamens and stigmas removed	21–3–21 to 26–3–21	2 white	40 30	None 1	Not tested
LARGE WELL DEVELOPED PLANTS VARYING IN MORPHOLOGICAL CHARACTERS NOTABLY IN THE COROLLA WHICH WAS WHITE, PINK AND WHITE SHADED WITH PINK		Do.	21–3–21 to 26–3–21	2 pink	41 31	None ,,	
		Stamens removed	21–3–21 to 26–3–21	2 white	30 *30	2 24	None 16 blown off by storm, 8 harvested gave good seed which germinated
		Do.	13–4–21	same plants*	7	4	None
		Do.	21–3–21 to 26–3–21	2 pink	50 24	None ,,	
1921–22	Original seed	Stamens and stigmas removed	12–3–22	1 pink	39	Very small *empty* capsules	
PLANTS NORMALLY DEVELOPED		Do.	24–3–22	1 parti-coloured	42	None	
		Do.	23–3–22	1 white	48	,,	

TABLE III—(*contd.*)

Season	Origin of culture	Nature of operation	Date	No. of plants and colour of corolla	No. of capsules per plant treated	No. of capsules which developed	No. of seed which germinated
1921-22 PLANTS NORMALLY DEVELOPED — *contd*	Original seed —*contd.*	Stamens removed	11-3-22	1 pink	40	Very small *empty* capsules	
		Do.		1 parti-coloured 1 white	50 40	None 7	None
	Seed from * gave pink, white and parti-coloured flowers	Stamens and stigmas removed	8-3-22	1 pink	33	None	
			10-3-22	1 parti-coloured	44	,,	
		Do.	8-3-22	1 white	51	,,	
		Stamens removed	8-3-22	1 pink	35	,,	
		Do.	11-3-22	1 parti-coloured	39	,,	
		Do.	8-3-22	1 white	50	,,	
1922-23	Seed of plants grown in 1922 from culture*						
	(1) Seed from white flowered plants	Stamens and stigmas removed	13-3-23	2	20 14	None ,,	
		Stamens removed	13-3-23	2	17 17	,, ,,	
	(2) Seed from pink flowered plants	Stamens and stigmas removed	13-3-23	1	20	None	
			18-3-23	1	20	,,	
		Stamens removed	13-3-23	2	34 17	None ,,	
	(3) Seed from parti-coloured flowers	Stamens and stigmas removed	1-4-23	1	9	None	
		Stamens removed	1-4-23	1	8	,,	

TABLE III—(*concld.*)

Season	Origin of culture	Nature of operation	Date	No. of plants and colour of corolla	No. of capsules per plant treated	No. of capsules which developed	No. of seeds which germinated
1922–23 —*contd.*	Seed of plants grown in 1921-22 from original seed						
	(1) Seed from pink flowered plants	Stamens and stigmas removed	1–4–23	1	8	None	
		Stamens removed	1–4–23	1	22	,,	
	(2) Seed from white flowered plants	Stamens and stigmas removed	13–3–23	1	20	,,	
			18–3–23	1	10	,,	
		Stamens removed	13–3–23	2	34 17	,, ,,	
	(3) Seed from particoloured flowers	Stamens and stigmas removed	1–4–23	1	9	,,	
		Stamens removed	1–4–23	1	8	,,	
	Seed of plants grown in 1920-21 culture 9	Stamens and stigmas removed	1–4–23	1 white	12	3	
		Stamens removed	1–4–23	1 white	8	1	
	Culture 6	Stamens and stigmas removed	3–4–23	1	13	None	
		Stamens removed	3–4–23	1	11	,,	
	Culture 410	Stamens and stigmas removed	19–3–23	2	12 11	None ,,	
		Stamens removed	19–3–23	2	15 12	,, ,,	

Although there was no *a priori* reason to suspect a correlation between the colour of the corolla and parthenocarpy, nevertheless the variously coloured flowers differed in their behaviour on this point. It will, therefore, be best to deal separately with the three classes, namely, those with pink, white and parti-coloured corollas.

Those with a *pink* corolla resembling *N. Tabacum* var. *Mirodato* will be considered first. In 1921, 146 buds (on 4 plants) gave no capsules. In 1921, two plants with 19 buds gave very small empty capsules and ten plants with 68 buds gave no capsules at all. A similar negative result was obtained in 1922-23.

In 1921-22, 175 parti-coloured buds (on 4 plants) were treated with negative results. In 1922-23 no plants with parti-coloured corollas gave fruits. Thus in the three years (1921 to 1923) no parthenogenesis occurred in plants with pink or parti-coloured corollas. Slight parthenocarpy was shown in plants with pink corollas but none in those with parti-coloured corollas.

The results obtained on plants with white corollas are somewhat different. Here undoubted parthenocarpy and phenospermy occurred. In 1921, out of four plants treated, one with 40 buds gave no capsules, the second with 30 buds gave one capsule, the third with 30 buds gave two capsules and the fourth produced no less than 24 capsules from 30 buds. The capsule given by the second plant was not examined. The two capsules of the third plant when tested gave no viable seed. Of the 24 capsules given by the fourth plant 16 were blown off in a storm and could not be examined. Eight, however, were left. These had entirely the appearance of normal capsules and contained good, sound, fertile seed. In view of the other results obtained this appeared so surprising that a month later seven additional buds were treated and four capsules were obtained. These, however, were the ordinary parthenocarpic fruits and contained no fertile seed. Seed from the eight apparently normal capsules was sown in the autumn of 1921. It germinated well and gave a large number of plants with variously coloured corollas. In 1922, 252 buds on six plants of this culture were treated but no capsules resulted. Similarly in 1923, 166 buds on ten plants from the following generation also gave no capsules. Thus no confirmatory evidence of the production in 1921 of parthenogenetic seed was obtained, either by subsequent castrations on the same plant or in succeeding years from the progeny raised from this supposed parthenogenetic seed. That the seed from a white flowered plant should have given rise to plants with variously coloured corollas is suggestive

of accidental cross-fertilization. White flowers are generally recessive. Too much stress cannot be laid on this point as no genetic analysis of the cross has been carried out. It appears improbable, however, that in the total absence of any other indication of parthenogenesis in these investigations eight well formed capsules should suddenly have been formed. On the other hand it seemed difficult at first to conceive of any accident likely to bring about such a result. It is nevertheless possible that pollen from a neighbouring branch was blown on to the newly castrated buds and this seems the most probable explanation. The junior author responsible for this operation was occasionally liable to interruption while at work. It would be quite sufficient to leave a newly castrated spray uncovered for a few minutes for pollination to be effected by the strong winds prevalent at Pusa in March.

Leaving this case aside and returning to the rest of the white flowered plants we find that six or eight plants showed parthenocarpy. In 1920 on three plants from 67 buds seven capsules were formed. In 1922, one out of two plants gave seven capsules from 40 buds and in 1922-23 the only two plants used gave four capsules from 20 buds. No fertile seed was obtained, the contents of the capsules consisting of empty seed coats. Thus in the hybrid culture, plants with parti-coloured flowers gave no indications of parthenocarpy or parthenogenesis, plants with pink flowers resembling var. *Mirodato* gave a very slight indication of parthenocarpy in one year only, while certain plants with white corolla like those of var. *Cuba* showed distinct parthenocarpy.

V. Summary.

The results obtained may be summed up as follows :—

(1) No evidence either of parthenogenesis or of parthenocarpy was obtained in connection with *Nicotiana Tabacum* var. *Mirodato* although the number of buds treated during the three seasons was over 1,100.

(2) *Nicotiana Tabacum* var. *Cuba* showed parthenocarpy but no parthenogenesis. All the plants treated in 1920-21 and 1921-22 gave capsules but no fertile seed.

(3) In the hybrid plants obtained from the cross between the above two varieties, a certain amount of parthenocarpy was observed. This was always associated with a white corolla (similar to that of the var. *Cuba* parent) but the converse, namely, that a plant with a white corolla should always show parthenocarpy did not hold. Whether this correlation is more than a coincidence cannot be definitely stated on account of the small number of plants employed.

(4) No evidence in favour of Goodspeed's suggestion that parthenogenesis might develop with acclimatization was obtained. All the evidence seems to point the other way. It was, however, found that large, vigorous plants gave a high percentage of parthenocarpic fruits whereas poorly grown plants of the same variety gave none at all. This would indicate that in any future experiments the nutrition of the parent plants should be one of the points considered. It might be possible to induce parthenogenesis by very heavy manuring and irrigation.

Under the conditions obtaining at Pusa, therefore, parthenogenesis could not be demonstrated in these two varieties of *Nicotiana*. Parthenocarpy was found to occur in var. *Cuba* but not in var. *Mirodato*.

PUSA,
July 3rd, 1923.

5. THE INHERITANCE OF CHARACTERS IN NICOTIANA RUSTICA L.

BY

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I. Introduction.

The importance of *Nicotiana rustica* L. to India is due to the fact that it will thrive in situations and localities which are unfavourable to *N. Tabacum*. The greater vigour of the seedlings, their rapid growth and the resistance to cold of the young plants are the chief factors which make this possible. Thus *N. rustica* is the tobacco almost exclusively grown in the Punjab, North-West Frontier Province and in the Western and Central Districts of the United Provinces where the low temperatures of December and January prevent the successful cultivation of *N. Tabacum*. For the same reason, *N. rustica* is cultivated in many of the colder parts of Europe, such as Germany and Russia where it would be impossible to grow *N. Tabacum* profitably. In Bihar, where both species do well, the proportion of *N. rustica* to *N. Tabacum* varies with the rainfall. In years with a heavy August rainfall, the seedlings of the more valuable *N. Tabacum* are very often destroyed. In such years, the more quickly growing *N. rustica* replaces *N. Tabacum* to some extent owing to its rapid development. Moreover, the seedlings of this species can be sown much later than those of *N. Tabacum*. The rapid growth of this species also accounts for its popularity in the villages of Eastern Bengal where it can be grown after the subsidence of the rain inundation of the monsoon period. It is possible that the faculty of *N. rustica* to establish itself rapidly and easily is partly connected with the size of the seeds which are much larger than those of *N. Tabacum*. It is evident that, although *N. rustica* is less valuable than *N. Tabacum*, it occupies a definite place in the tobacco cultivation of India. In certain localities

it cannot be replaced by *N. Tabacum*. In improving the tobacco crop, therefore, both species will have to be considered but the improvement to be aimed at will be somewhat different in each case. In *N. Tabacum* the texture of the cured leaf is very important. In yellow flowered tobacco, the leaves are always thick and coarse and are used for purposes such as snuff in which these characters are of minor significance. The improvements which must be aimed at are increased yield and vigour and better flavour.

Hybridization experiments on both species of tobacco (*N. Tabacum* and *N. rustica*) were begun in 1908-09 with the object of obtaining information on the mode of inheritance of the various characters. The work has proved more onerous than was expected on account of the numerous factors involved and the large number of plants which this necessitates. Considerations of time and space have, therefore, made it imperative to concentrate on one species only, and, as *N. Tabacum* is the more valuable crop, this has been selected for further work. The results obtained up to the present with *N. rustica* are, however, of some interest and may prove of use to future workers. As in the case of *N. Tabacum*, the characters are very complex and the factors involved are numerous. In this paper, date of flowering, height, length of the pistil and stamens, form of the inflorescence, and the undulation of the leaf margin are the characters considered.

II. The Occurrence of Parthenogenesis.

The possible formation of parthenogenetic seed in various species of *Nicotiana* has been investigated by many workers.[1] In no case has either parthenogenesis or parthenocarpy been shown to occur in *Nicotiana rustica*. Experiments with this particular species have, however, not been very numerous. It was, therefore, considered advisable to make a detailed study of the Indian varieties with regard to this point.

A large number of experiments on parthenogenesis were made in Pusa in 1910 and 1911. The conditions were as varied as could be devised and all the twenty Indian types[2] were employed. These include races in which self-fertilization is normally impossible—such as Type 1—as it seemed possible that apogamy might occur more easily in such types. Castration of the flowers is easy but it is necessary to remove the stamens at an early stage as the anthers often burst before the flowers open. In some cases, the anthers

[1] Wellington, R., *Amer. Nat.*, Vol. 47, 1913, p. 279.

[2] Howard, A., and Howard, G. L. C., Studies in Indian Tobaccos No. 1. The types of *Nicotiana rustica* L. *Mem. of the Dep. of Agr. in India, Botanical Series*, Vol. III, 1910, p. 1.

only were removed, in others both stigma and anthers. Young plants, plants in full maturity and plants which were almost over but still capable of forming seed, were used. In some individuals, side branches were chosen, in others the main inflorescence. The result, in all cases, was the same. The corolla remained fresh for an abnormally long time, the ovary began to grow out and then dropped. On examination, this enlarged ovary was found to contain no seeds. Sometimes, the young ovaries were left exposed to the air after the corolla had fallen, but generally they were protected against accidents by covering the spray with a perforated bag. In all cases, however, the immature capsules were shed when less than half grown. An abnormal development of new buds and flowers was observed on castrated inflorescences, whereas in inflorescences in which flowers have been cross-pollinated, only a limited number of buds were formed. This would indicate infertility of the castrated ovaries and a consequent stimulus to the plant to produce effective capsules. In 1910, one or two plants of each type (30 to 40 plants in all), were employed; in 1911, certain selected types only. The results of 1911 are given in Table I.

TABLE I.

Experiments on the production of parthenogenetic seeds (1911).

Type number	Normal method of pollination	Condition of the plant	Number of buds castrated	Number of capsules obtained
Type 4	Cross & self	In full flower	50	*Nil*
„	„	„	50	*Nil*
„ 5	Self	In full flower and fruit	38	*Nil*
„ 6	Cross	Nearly over	42	*Nil*
„ 8	Cross & self	Three-quarters over	42	*Nil*
„ 11	Cross & self	Three quarters over	34	*Nil*
„ 12	Cross & self	..	39	*Nil*
„ 16	Cross & self	In full flower and fruit	22	*Nil*
„ 17	Cross & self	In full vigour	50	*Nil*
„ 18	Cross & self	Fairly young	13	*Nil*
F_1 Type 1 × Type 18	Cross & self	In full vigour	36	*Nil*
11 plants			421 buds	No capsules

In both 1910 and 1911, not a single capsule or seed was formed and no evidence either of parthenogenesis or parthenocarpy could be found among the twenty Indian types when grown at Pusa.

III. The Experimental Results.

The importance of absolutely uniform environmental conditions in investigations dealing with the inheritance of the size of organs has been emphasized in a previous paper[1] on the genetics of *N. Tabacum*. The precautions, described in that paper, were observed in the present series of experiments and similar methods of raising the experimental plants were employed. The six parents were selected from among the twenty types previously described.[2] These had been under observation in the experimental area for a number of years. All the seed employed, whether of parents or of hybrids was raised under parchment bags. The actual operation of hybridization is easy in this species but care must be taken to remove the stamens at an early stage as, in many of the types, the anthers burst in the bud. To remove the stamens effectively, it is necessary to slit the corolla on one side. In India, crossing can be most successfully effected if the buds are castrated in the evening and if the stigmas are pollinated during the following day. Want of space and time have, unfortunately, kept the number of plants under observation smaller than was desirable, especially in the case of the parents. The range of variation in these was determined each year afresh.

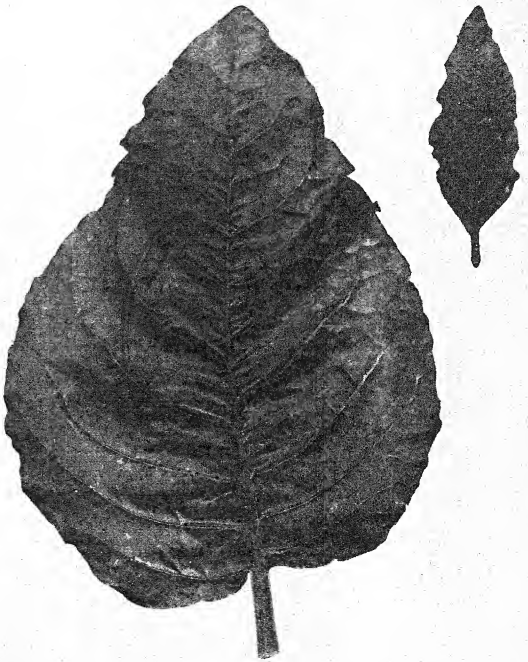
The following crosses were made :—

(1) Type 1 (tall) × Type 5 (tall)
(2) Type 1 (tall) × Type 18 (short)
(3) Type 1 (tall) × Type 16 (short)
(4) Type 16 (short) × Type 15 (short)
(5) Type 16 (short) × Type 17 (short).

The full descriptions of these types will be found in the Appendix (p. 35).

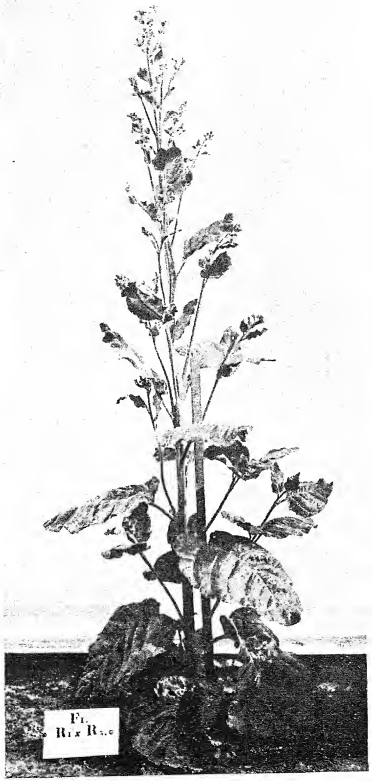
In all the characters except height, the F_1 was intermediate between the two parents, in most cases occupying the position of the exact average between them. These results agree with those previously obtained in the case of *N. Tabacum*[3, 4, 5]. As regards the height, however, in all crosses, whether tall × tall, tall × short or short × short, the F_1 plants were distinctly taller than either parent. On this point the results differ from those obtained previously with *N. Tabacum* L. where the F_1 was often intermediate in height generally with a leaning towards the taller parent[3, 4, 5]. The types used in

[1] Howard, G. L. C., *Mem. of the Dept. of Agr. in India, Botanical Series*, Vol. VI, 1913, p. 5
[2] Howard, A., and Howard, G. L. C., *l.c.*
[3] Hayes, H. K., Bulletin 171, *Connecticut Agr. Exp. St.*, 1912.
[4] Howard, G. L. C., *l.c.*
[5] Setchell, W. A., Goodspeed, T. H., and Clausen, R. E., *University of California Publications in Botany*, Vol. V, 1922, p. 458.



TYPE 1.

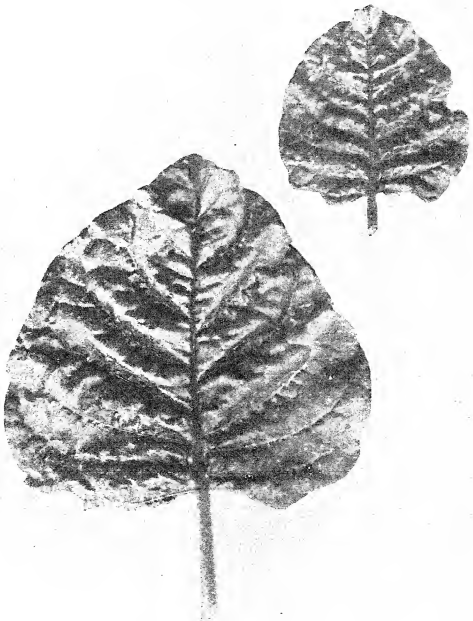
TYPE 5.



F1.
R1 x R5.

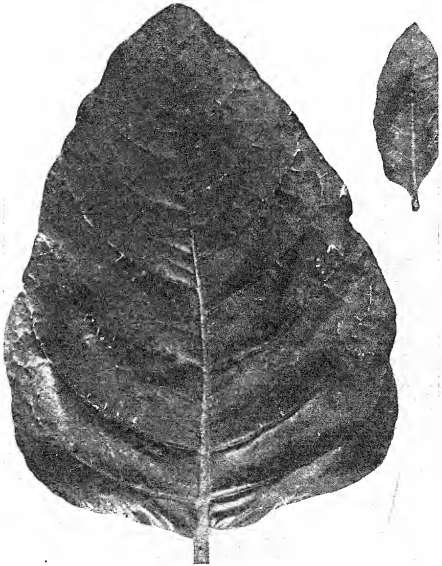
PLATE III.

F, TYPE 1 × TYPE 5.



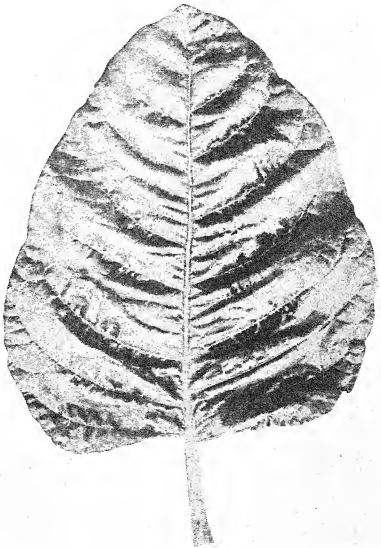
TYPE 15.

PLATE IV.



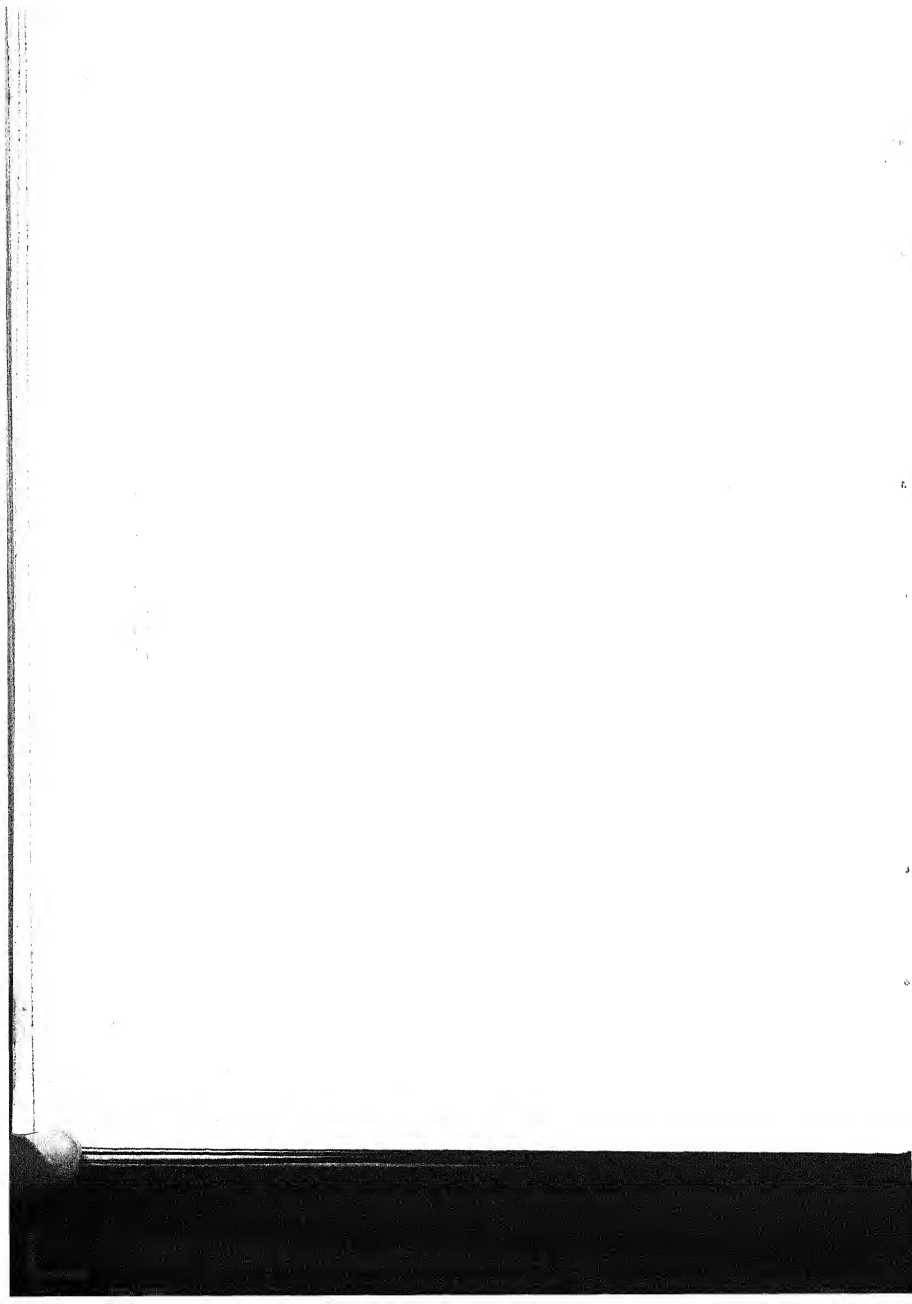
TYPE 16.

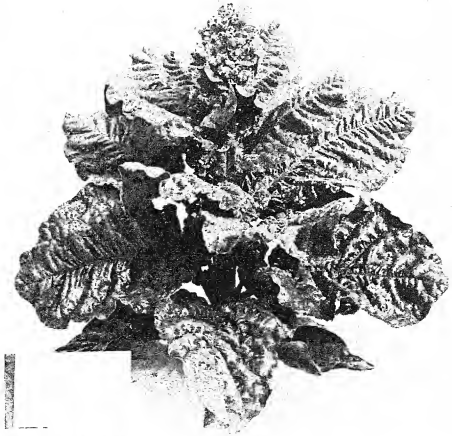
PLATE V.

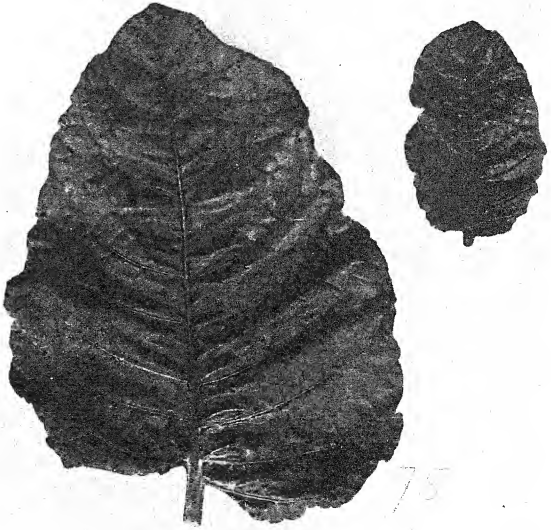


F1
R16 x R15

F, TYPE 16 × TYPE 15.

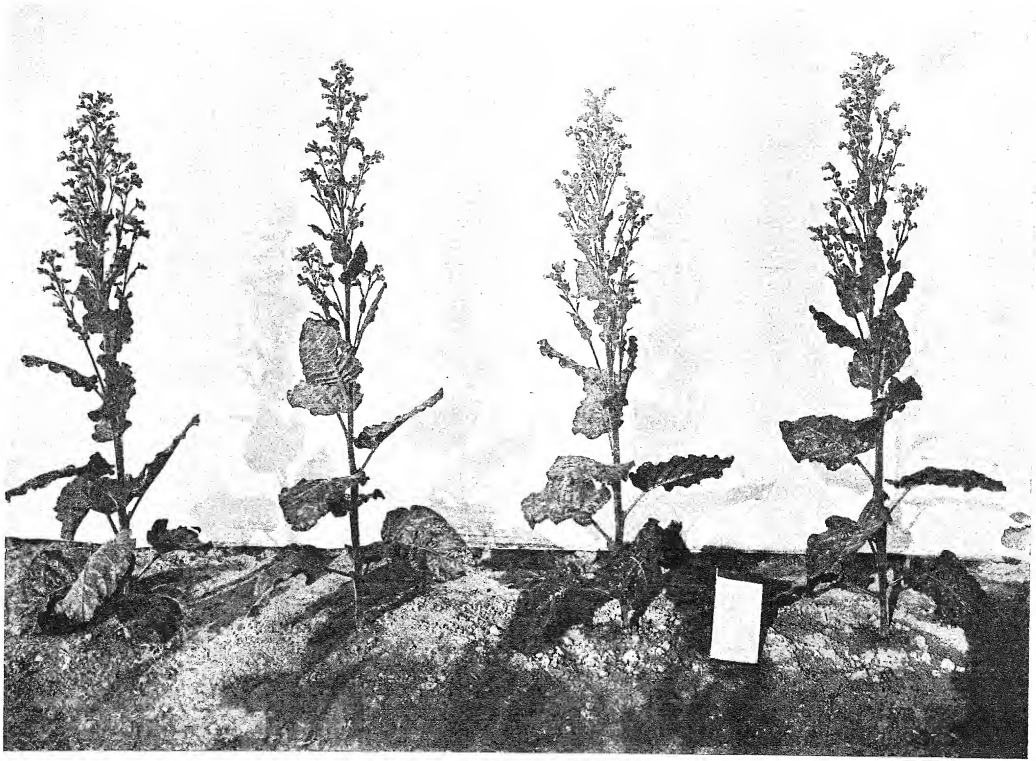






TYPE 17.

TYPE 18.



F_1 TYPE 1 × TYPE 18.

PLATE IX.

Table II.

Time of Flowering.

	Date of flowering of parent	December			January																February										Total Number of plants
		26-27	28-29	30-31	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	
Type 1	..	..	..	..	..	..	..	5	82	32	39	54	55	47	35	18	10	5	7	2	2	..	..	..	..	..	..	..	..	..	393
Type 18	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	3	4	17	23	21	10	1	..	..	..	..	..	..	..	81
F_1 Type 1 × Type 18	..	..	..	..	..	..	..	..	..	..	..	1	..	6	17	15	7	2	2	1	..	1	..	..	..	..	..	..	..	..	52
F_2 Type 1 × Type 18	..	..	..	..	..	..	3	5	10	20	33	29	71	75	74	100	67	61	54	39	25	11	1	1	3	..	4	..	..	..	686
F_1 (Type 1 × Type 18) × Type 1 ..	..	..	..	..	..	1	2	3	6	15	28	37	39	40	44	48	40	20	11	9	4	1	..	..	..	..	..	..	..	..	348
F_1 (Type 1 × Type 18) × Type 18 ..	..	..	..	..	..	..	..	..	..	..	..	..	4	7	12	45	32	39	39	25	18	2	4	2	1	1	..	..	..	..	231
Type 16	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	3	7	10	3	6	2	3	..	1	1	..	..	..	..	..	39
Type 17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	2	2	4	6	1	1	..	1	1	20
F_1 Type 16 × Type 17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	4	13	16	11	15	10	3	9	8	3	..	2	..	..	95
Type 16	..	..	..	..	..	..	..	..	..	..	..	1	5	8	26	25	34	29	18	13	4	4	2	1	..	..	..	..	..	..	170
Type 17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	15	15	8	6	1	3	2	..	..	..	52
F_2 Type 16 × Type 17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	5	16	28	54	41	26	17	2	7	2	1	2	..	..	..	202
Type 1	..		..		..	..	..	..	1	2	8	6	7	7	1	2	..	1	1	..	..	..	..	..	..	..	..	..	..	..	36
Type 5	..	..	..	..	..	..	..	..	..	..	..	1	3	5	3	3	1	1	..	1	1	..	..	..	..	..	..	..	..	..	19
F_1 Type 1 × Type 5	..	..	..	..	..	..	..	..	..	..	3	7	16	10	13	4	2	1	..	..	..	..	..	..	..	..	..	..	..	..	56
Type 1	..	..	..	..	..	..	..	..	5	12	32	39	54	55	47	35	18	10	5	7	2	2	..	..	..	..	..	..	..	..	323
Type 5	..	..	..	..	..	..	..	2	16	22	27	42	30	34	13	4	..	5	..	..	..	..	..	..	..	..	..	..	..	..	204
F_2 Type 1 × Type 5	..	4	1	2	6	22	20	56	57	62	78	74	96	107	77	45	34	15	9	6	16	4	3	1	..	..	..	..	..	..	795
Type 15	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	8	7	6	4	4	4	..	1	..	..	..	..	..	..	38
Type 16	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	3	7	10	3	6	2	3	..	1	1	..	..	..	..	..	39
F_1 Type 15 × Type 16	..	..	..	..	..	..	..	..	..	..	..	..	..	4	5	13	23	3	4	11	5	2	2	1	..	..	..	..	..	..	73
Type 1	..	..	..	..	..	..	..	..	..	1	2	8	6	7	7	1	2	..	1	1	..	..	..	..	..	..	..	..	..	..	36
Type 16	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	3	7	10	3	6	2	3	..	1	1	..	..	..	..	..	39
F_2 Type × Type 16	..	..	..	..	..	..	..	..	2	51	20	31	60	46	62	79	71	46	34	23	17	10	6	3	3	..	..	..	..	..	564
Type 1	..	..	..	..	..	..	..	5	12	32	39	54	55	47	35	18	10	5	7	2	2	..	..	..	..	..	..	..	..	..	323
Type 16	..	..	..	..	..	..	..	..	..	..	..	1	5	8	26	25	34	29	18	13	4	4	2	1	..	..	..	..	..	..	170
F_1 Type 1 × Type 16	..	..	..	..	..	..	..	..	..	..	5	14	46	46	48	34	16	8	5	2	1	1	..	..	..	..	..	..	..	..	226
F_2 Type 1 × Type 16																															
440	21st January	2	2	2	2	9	5	9	4	8	8	8	4	5	3	6	..	1	..	..	..	..	..	..	..	..	..	..	..	..	78
61	19th „	..	..	..	1	5	6	11	10	20	16	24	31	29	23	28	24	12	10	8	1	1	..	..	..	..	..	..	..	..	260
182	15th „	..	..	..	..	..	..	9	9	25	36	21	26	20	8	7	4	2	..	1	..	..	..	..	..	..	..	..	..	..	168
127	26th „	..	..	..	..	..	..	3	1	6	5	9	13	17	16	19	11	6	3	2	2	1	..	..	..	..	..	..	..	..	114

this investigation and some of the first generations are shown in Plates I to IX.

1. *Time of flowering.*

The date of the opening of the first flower on each plant was taken as the criterion of this character. The examination was carried out each morning at approximately the same time and the number of plants flowering for the first time recorded. The absence of rain and the generally settled and uniform character of an Indian cold season make such observations easier and more trustworthy than they would be in a climate like Europe, where cloud and absence of sun might introduce irregularities. The observations are given in Table II.

Unfortunately, on account of illness, these observations could be carried on to the F_2 in one case only, namely, in the cross Type 1 × Type 16. The results are very similar to those observed in the case of *N. Tabacum*[1]—the F_1 is intermediate between the two parents and the F_2 shows a range greater than the combined ranges of the parents. In the F_3, segregation takes place and the range of variation of the progeny of each F_2 plant is different. As would be expected from the fact that the range of the F_2 generation exceeds that of the parents, some of the plants in the F_3 are distinctly earlier than either parent, for example No. 440.

In Type 1 × Type 5, we have another case which the range of the F_2 is much greater than that of the parents. The number of early plants in this case is especially marked.

2. *The leaf characters.*

The leaves of all the Indian types of *N. rustica* are characterized by a great unevenness or puckering of the surface, due to irregularities in the growth of the lamina between the veins. In some types, such as 17 and 18, this puckering is very marked while in Type 16 the leaves are almost but not quite flat. All stages of intensity occur among the various types but these undulations are never absent even in Type 16. In addition to this uneven puckering of the surface, there is in some types a very definite regular undulation or frilling of the margin of the leaf. This frilling of the edge is quite distinct from and is inherited separately to the undulations of the general surface. No attempt was made to determine the mode of inheritance of the surface undulations but observations were made on the relation between the frilled and flat margins in leaves. The two types used in determining the

[1] Howard, G. L. C., *l.c.*

inheritance of this character were Type 1 (Plate I) with a wavy or undulated edge and Type 16 (Plate V) in which the edge is practically flat. A few undulations appear in the photograph but these are due to the uneven growth of the lamina itself, whereas the slight undulation or frilling in Type 1 is confined to the margin. The F_1 was intermediate between the two parents; the margin was wavy but less so than in Type 1. In the F_2, plants with frilled and flat margins were found in the following proportions:—

Total No. of plants	Wavy margin	Flat margin
557	424	133
	Ratio 3·3	1

In the F_3, a large number of plants were grown. Those in which the margin of the leaf was flat, invariably gave progeny with flat-margined leaves. Some of those in which the leaf margin was undulated bred true to this character. Others gave rise to a mixed progeny consisting of plants with frilled and flat margins in the ratio of approximately 3 : 1.

It would, therefore, appear that there is only one factor involved in the undulation or frilling of the margin and that a flat edge is recessive to a wavy margin. A similar result was obtained in the case of *N. Tabacum*.[1]

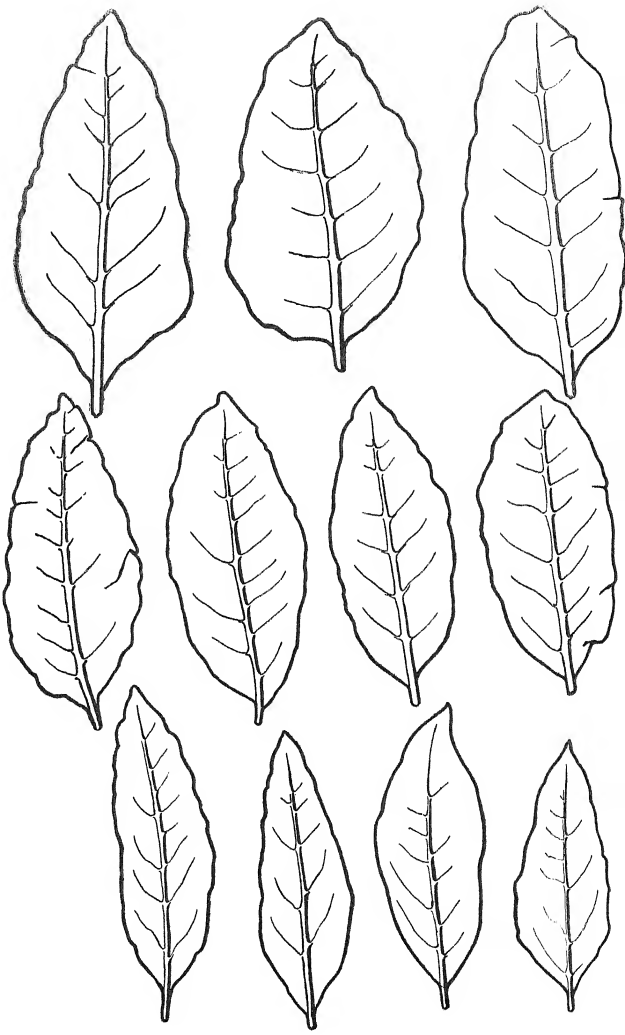
The shape of the leaf has not been investigated in any great detail. It will be seen from Plates I to IX that the leaves of the F_1 are generally intermediate in shape and size between those of the parents and this is particularly well shown in the inflorescence leaves. In the F_2, a series is obtained in which the general outline, the size and the apex all vary. In Plate X, typical representatives of the F_2 series in the cross Type 1 × Type 16 are shown.

3. *The flower characters.*

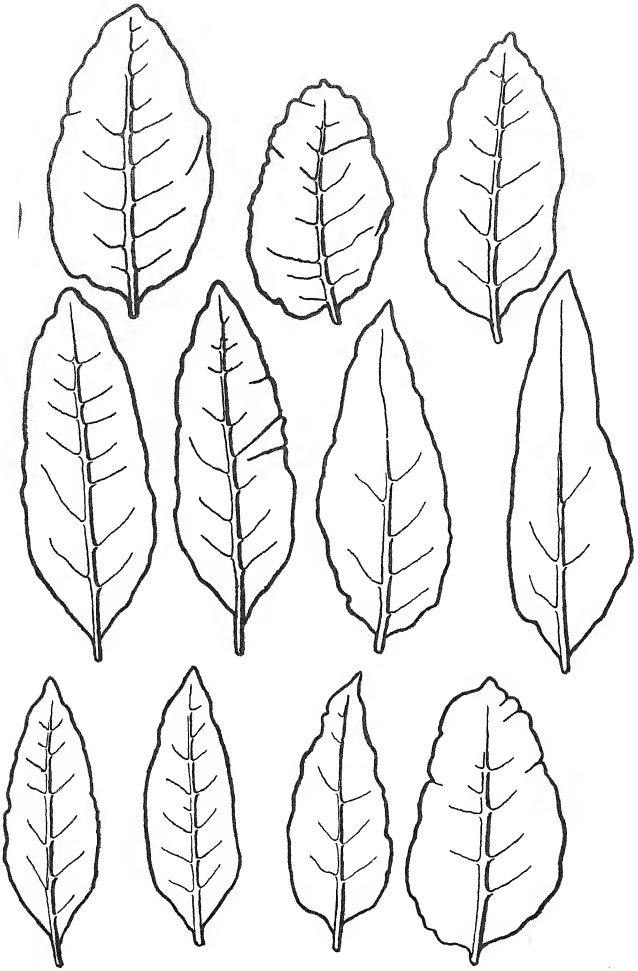
As regards the flower characters, the F_1 between any two types is generally a perfect intermediate between the parents. In Plate XI the parents and the F_1 in two crosses Type 1 × Type 18 and Type 15 × Type 16 are shown. In the shape of the sepals, the shape and size of the corolla, the shape of the capsules and the position of the essential organs, the F_1 is half-way between the two parental types.

The first point investigated was the relative lengths of the pistil and stamens. In *N. rustica*, the stamens are adherent to the corolla and the

[1] Howard, G. L. C., *l.c.*



LEAFSHAPE IN THE F_2 OF TH



HE CROSS TYPE 1 × TYPE 16.

relative position of the anthers to the stigma varies in the different types.[1] Thus the anthers may be below the stigma, at the same level as the stigma or above it. In observations on this character, it is best to carry out the examination of the flowers in the field. Care must be taken to use only those flowers which are borne on the main inflorescence and only those flowers which open during the real flowering period.[2] In late-formed flowers or in flowers on late-formed side branches, the relative position of the stigma and anthers varies considerably. This phenomenon is seen in all the types. The general tendency is for the later formed flowers to have both anthers and stigmas at the same level. The stage in the development of the individual flower is also of importance as the relative positions of the anthers and stigma change during development. It is advisable to use flowers which have just opened fully and to carry out the examination in the morning when such flowers are abundant.

The first cross investigated was that of Type 1 × Type 16. In Type 1, the anthers are far below the stigma, rendering self-fertilization, except by external agency, impossible. In Type 16, the anthers and stigma are approximately at the same level. The F_1 is intermediate in character, the anthers being just below the stigma as in the first generation of Type 1 × Type 18 (Plate XI). In the F_2, plants resembling both parents and also the first generation occurred. It was possible to separate those forms which resemble Type 16—those in which the anthers were at the same level as the stigma—from the remainder. It was not possible, however, to distinguish between the forms resembling Type 1 and those which were like the first generation. The following numbers were obtained in an examination of two F_2 cultures :—

TABLE III.

Relative position of the anthers and stigma in the F_2 of Type 1 × Type 16.

Total No. of plants	No. of plants with anthers below stigma	No. of plants with anthers level with stigma
528	393	135
518	382	136
1046	775	271
RATIO ..	2·9	

[1] Howard, A. and Howard, G. L. C., *l.c.*

[2] East, E. M., *Amer. Jour. of Botany*, Vol. 3, 1916, p. 210.

Five plants were grown in the F_3 generation. The results are given below :—

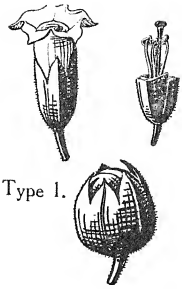
TABLE IV.

Relative position of the anthers and stigma in the F_3 generation of Type 1 × Type 16.

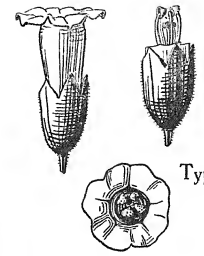
Culture	Parent	F_3 generation
A 61	Anthers below the stigma	182 plants with the anthers below the stigma ; 76 plants with the anthers level with the stigma
A 127	Anthers below the stigma	120 plants, all with the anthers below the stigma
A 182	Anthers below the stigma	150 plants, all with the anthers below the stigma
A 440	Anthers level with the stigma	90 plants, all with the anthers level with the stigma
A 490	Anthers level with the stigma	180 plants, all with the anthers level with the stigma

The F_4 progeny of two plants of A 127 were examined and, in all cases, the anthers were well below the stigma. It would thus appear that the difference in the relative positions of the anthers and stigma in Type 1 and Type 16 is due to the existence of only one factor. Measurements were made of the length of the pistil, of the corolla tube and of the height of the anthers above the base of the corolla. It was found that the length of the corolla tube and the height of the anthers above the base of the flower was the same in both types but that the height of the stigma above the base of the corolla was different. If this difference in the length of the pistil is caused by a single factor, the results obtained in the cross Type 1 × Type 16 are explained.

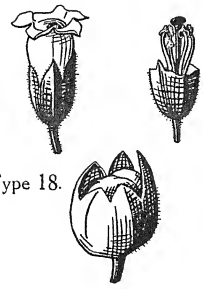
The next case investigated was that of the cross Type 1 × Type 5. As stated above, in Type 1 the anthers are well below the stigma. In Type 5, on the other hand, they are markedly above it and their relative position with regard to the stigma resembles that in Type 15 in Plate XI. In the F_1, the anthers were slightly above the stigma. In the F_2, a series was obtained with every transition between the two parent types. A few plants resembled Type 5 and a few Type 1 but, in most, the anthers were either just below the stigma, just above it, or at the same level. Measurements of the flowers showed that, not only was there a difference between the height of the stigmas above the base of the corolla, but that the height of the anthers and of the



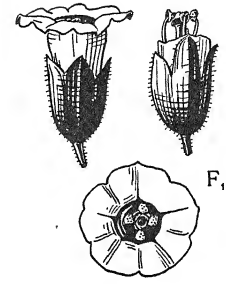
Type 1.



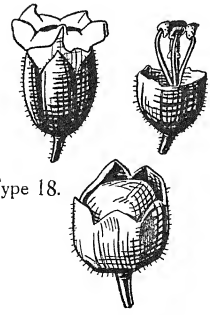
Type 15.



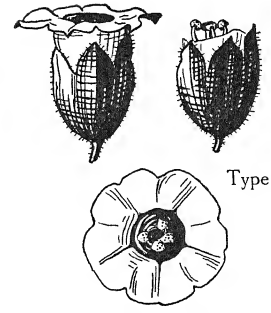
F_1 Type 1 × Type 18.



F_1 Type 15 × Type 16.



Type 18.



Type 16.

FLORAL DETAILS IN TYPE 1 × TYPE 18 AND IN TYPE 15 × TYPE 16.

length of the corolla varied also. In Type 1, the height of the anthers above the base of the corolla is 16 to 16·5 mm., in Type 5, 15 to 15·5 mm. Here, therefore, at least two factors are concerned. This explains the series obtained in the F_2. As the differences involved are small and the errors of measurement considerable, this point was not followed any further.

The other flower characters considered were the diameter of the calyx and corolla. The diameter of the calyx at its widest point was taken and the diameter of the corolla was measured just below the point of expansion of the corolla tube to form the limbs. It will be seen from Platè XI that Type 1 and Type 16 differ very markedly in these characters. As mentioned above, the length of the corolla tube is the same in both. Six typical flowers were measured on each plant of the F_2. The results agree generally with those obtained for other species of *Nicotiana* by several observers. The range in the F_2 covered the combined range of both parents. The work was not carried any further owing to the difficulty and labour involved in obtaining accurate measurements of such small differences.

TABLE V.

Inheritance of the maximum diameter in corolla and calyx.

Diameter of the corolla.

Type 1 .. .. Average diameter .. 12·0 mm. Range 11·5 mm. to 12·5 mm.

Type 16 .. .. „ „ .. 14·0 mm. „ 13·5 mm. to 14·5 mm.

	F_2 Type 1 × Type 16						
Diameter in mm. ..	11·5	12·0	12·5	13·0	13·5	14·0	14·5
No. of plants ..	16	42	66	41	25	12	7

Diameter of the calyx.

Type 1 .. .. Average diameter .. 12·5 mm. Range 11·5 mm. to 13·0 mm.

Type 16 .. .. „ „ .. 17·5 mm. „ 16·5 mm. to 18·0 mm.

	F_2 Type 1 × Type 16.							
Diameter in mm. ..	11·5	12·0	12·5	13·0	13·5	14·0	14·5	15·0
No. of plants ..	2	9	16	20	30	28	21	22

It will be seen that the range in the F_2 covers the combined range of both parents but is no greater.

4. *Height of the plant and form of the inflorescence.*

It will be necessary to consider these two characters together as the form of the inflorescence materially affects the height of the plant. The

Indian types of *N. rustica* fall into two groups which are easily distinguished in the field (1) tall plants with an open habit and long internodes, (2) short plants with short internodes. That the difference in height between these two groups is due to the length of the internodes and not to their number is shown by the following Table :—

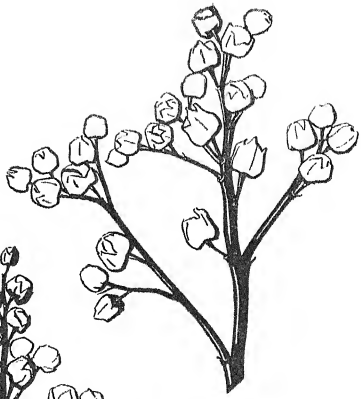
TABLE VI.

No. of internodes (stem)[1] *and height (1911).*

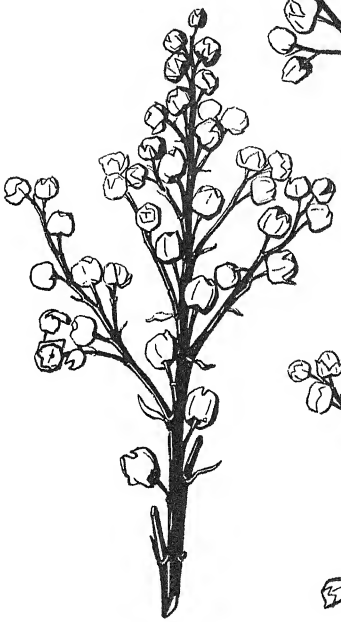
	Type	No. of internodes		Average height
		Average	Range	cm.
Group I (tall)	.. Type 1	28·5	27 to 31	175·3
	Type 5	25·5	23 to 28	154·9
	F_1 Type 1 × Type 5 ..	27·5	26 to 30	216·0
Group II (short)	.. Type 16	28·0	26 to 30	101·4
	Type 17	30·0	29 to 32	102·5
	F_2 Type 16 × Type 17..	29·5	26 to 32	111·7
	Type 15	27·5	25 to 31	95·8

In the first group not only are the internodes of the leafy portion of the stem long but the inflorescence is also extended with long internodes. In the second group, the internodes of the stem are always short but the inflorescence may be extended and open (Plate XV) or very compact (Plate VII). There appear to be a number of intermediates between these two extremes. In addition to this difference in the length of the internodes of the axis of the inflorescence, the flowers themselves may be borne at long or short intervals on the branches thus giving an appearance of sparse or crowded flowering. Plate XII, on which branches of certain types are drawn to scale, illustrates this point. A plant with long internodes generally has the capsules far apart but this is not always the case. For instance, the proximity of the capsules in Type 5 and in Type 16 is much alike although the form of the inflorescence, depending on the length of the internodes on the main stem, is very different (Plates XII, II and V).

[1] The word stem is used to denote the leaf-bearing axis of the plant as opposed to the axis of the inflorescence.

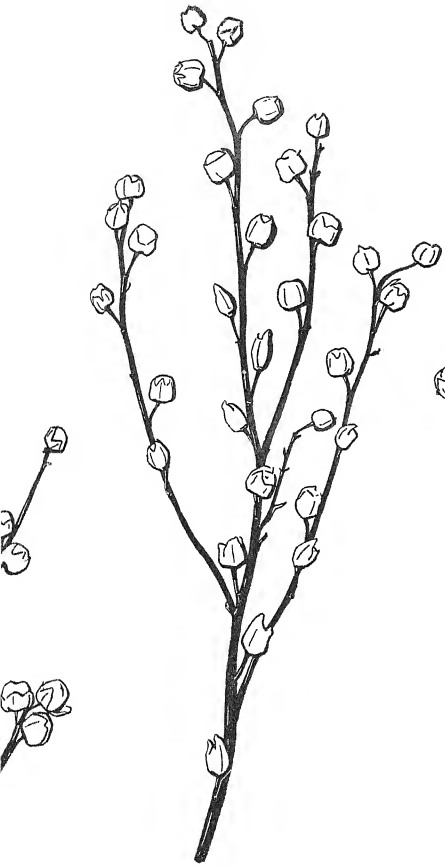


Type 16.

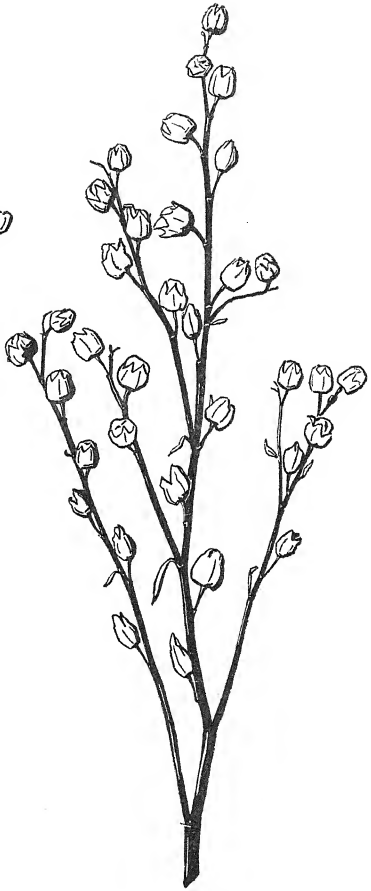


Type 18.

Type 5.



F, Type 1 × Type 18.



Type 1.

BUTION OF CAPSULES.

TABLE VII.

Height Type 1 × Type 18.

	Height of parent	CENTIMETRES																																Total No. of plants	Mean	S. D.	C. of V.
		77	82	87	92	97	102	107	112	117	122	127	132	137	142	147	152	157	162	167	172	177	182	187	192	197	202	207	212								
Type 18	..	1	2	8	13	19	19	4	7	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	76	98·4 ± ·68	8·8 ± ·62	8·9 ± ·61
Type 1	..	..	..	..	..	..	..	..	..	1	3	11	17	23	23	22	16	11	4	1	..	..	..	..	..	..	..	..	..	132	142·0 ± ·60	10·3 ± ·43	7·2 ± ·30
F_1 Type 1 × Type 18 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	3	1	3	13	5	7	5	3	6	2	..	..	49	177·5 ± ·54	5·7 ± ·39	3·2 ± ·21
F_2 Type 1 × Type 18 ..	..	1	..	6	7	10	13	18	17	12	9	9	11	5	14	27	23	32	43	51	44	30	33	18	16	11	9	4	1	474	155·0 ±1·58	29·2 ±1·12	18·8 ± ·72
Type 1 × F_2 (Type 1 × 18) ..	..	..	..	..	..	..	..	..	..	2	11	8	8	17	32	33	41	31	19	18	5	6	1	..	..	..	..	..	..	232	149·4 ± ·58	13·15 ± ·41	8·80 ± ·27
Type 18 × F_2 (Type 1 × 18) ..	..	1	4	4	6	8	14	9	9	1	..	1	1	..	..	2	9	5	14	5	7	6	2	4	1	2	..	2	..	117	135·0 ±2·24	35·96 ±1·58	26·64 ±1·17
Type 18	..	..	..	3	10	37	52	49	23	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	175	102·9 ± ·29	5·92 ± ·21	5·75 ± ·20
Type 1	..	..	..	..	..	..	..	..	..	..	2	3	5	11	10	17	20	21	8	2	..	..	..	..	..	..	..	..	..	99	148·2 ± ·68	10·05 ± ·48	6·78 ± ·32
F_2 Type 1 × Type 18 ..																																					
138	96	..	1	12	22	50	39	15	2	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	144	98·6 ± ·35	6·40 ± ·25	6·49 ± ·26
7	114	..	..	10	22	31	38	29	9	3	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	143	100·5 ± ·40	7·21 ± ·29	7·17 ± ·28
3	133	..	1	..	7	20	22	29	38	18	12	5	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	153	105·8 ± ·51	9·43 ± ·36	8·91 ± ·34
153	125	..	2	13	12	16	28	20	17	10	8	6	5	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	138	105·5 ± ·70	12·23 ± ·49	11·59 ± ·47
229	123	..	..	2	17	23	37	31	22	13	4	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	162	98·3 ± ·55	10·39 ± ·38	10·57 ± ·39
100	113	..	..	..	..	1	3	16	27	27	37	11	10	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	137	118·4 ± ·48	8·37 ± ·34	7·07 ± ·28
221	133	..	..	..	6	3	13	23	29	31	30	20	12	7	8	5	1	1	..	..	..	..	..	..	..	..	..	..	..	189	118·7 ± ·62	12·81 ± ·44	10·79 ± ·37
58	147	..	..	..	..	..	..	2	7	16	28	26	32	18	14	4	..	..	..	..	..	..	..	..	..	..	..	..	..	147	128·3 ± ·49	8·94 ± ·35	6·98 ± ·27
312	144	..	..	..	..	..	..	..	5	12	15	23	29	29	25	9	5	2	1	..	..	..	..	..	..	..	..	..	..	155	133·0 ± ·54	10·15 ± ·38	7·63 ± ·29
125	193	..	..	3	7	3	6	9	6	11	2	1	3	4	7	1	4	5	14	5	15	22	13	12	11	3	4	3	1	175	155·0 ±1·76	34·80 ±1·25	22·45 ± ·80
116	206	..	..	..	..	..	1	1	10	11	12	5	5	4	5	4	4	4	10	11	17	24	13	20	13	10	10	4	..	198	164·2 ± 1·3	28·56 ± ·96	17·39 ± ·58

	192	197	202	207	212	Total No. of plants	Mean	S. D.	C. of V.
Type 18	..	..	..	..	..	76	98·4 ± ·68	8·8 ± ·62	8·9 ± ·61
Type 1	..	..	..	..	..	132	142·0 ± ·60	10·3 ± ·43	7·2 ± ·30
F_1 Type 1 × Type 18	3	6	2	..	..	49	177·5 ± ·54	5·7 ± ·39	3·2 ± ·21
F_2 Type 1 × Type 18	9	11	9	4	1	474	155·0 ± 1·58	29·2 ± 1·12	18·8 ± ·72
Type 1 × F_2 (Type 1 ×	..	..	..	..	..	232	149·4 ± ·58	13·15 ± ·41	8·80 ± ·27
Type 18 × F_1 (Type 1 ×	1	2	..	2	..	117	135·0 ± 2·24	35·96 ± 1·58	26·64 ± 1·17
Type 18	..	..	..	..	..	175	102·9 ± ·29	5·92 ± ·21	5·75 ± ·20
Type 1	..	..	..	..	..	99	148·2 ± ·68	10·05 ± ·48	6·78 ± ·32
F_2 Type 1 × Type 18									
	..	..	..	..	..	144	98·6 ± ·35	6·40 ± ·25	6·49 ± ·26
	..	..	..	..	..	143	100·5 ± ·40	7·21 ± ·29	7·17 ± ·28
	..	..	..	..	..	153	105·8 ± ·51	9·43 ± ·36	8·91 ± ·34
	..	..	..	..	..	138	105·5 ± ·70	12·23 ± ·49	11·59 ± ·47
	..	..	..	..	..	162	98·3 ± ·53	10·39 ± ·38	10·57 ± ·39
	..	..	..	..	..	137	118·4 ± ·48	8·37 ± ·34	7·07 ± ·28
	..	..	..	..	..	189	118·7 ± ·62	12·81 ± ·44	10·79 ± ·37
	..	..	..	..	..	147	128·3 ± ·49	8·94 ± ·35	6·98 ± ·27
	..	..	..	..	..	155	133·0 ± ·54	10·15 ± ·38	7·63 ± ·29
	1	3	4	3	1	175	155·0 ± 1·76	34·80 ± 1·25	22·45 ± ·80
	3	10	10	4	..	198	164·2 ± 1·3	28·56 ± ·96	17·39 ± ·58

Five crosses were made to investigate the factors concerned in the height:—

(1) Type 1 × Type 16.
(2) Type 1 × Type 18.

These two are examples of a cross between a tall plant with a very open inflorescence (Type 1) and a short plant in which the inflorescence is crowded (Types 16 and 18, the inflorescence of Type 16 being a little more open than that of Type 18).

(3) Type 1 × Type 5.

This represents the union of two tall races, Type 5 being shorter and having a slightly more crowded inflorescence than the other.

(4) Type 16 × Type 17.
(5) Type 16 × Type 15.

Types 16 and 17 are both short (81 and 72 cm. in 1908) with crowded inflorescences. In the last cross, both parents are short (81 and 82 cm.) but in Type 15 the inflorescence is more open.

The height of the F_1 was, in all cases, greater than that of the tallest parent. In some cases this difference was small in amount; in others, notably in Type 1 × Type 5, it was relatively much greater.

Short × *short.*

The case of the cross *short* × *short* (Type 16 × Type 17 and Type 16 × Type 15) will be considered first. These three types do not differ greatly in height. As stated above, in both cases the F_1 was distinctly taller than either parent. A large number of plants were grown in the F_2 generation. All were short; nothing taller than the F_1 was produced in either cross. No extensive series of measurements were made but the F_2 cultures appeared almost uniform and very like the parents. Thus, in these two cases *short* × *short* gave only short plants of approximately the same height as that of the parents and the F_1.

Tall × *short.*

The union of *tall* × *short* (Type 1 × Type 18 and Type 1 × Type 16) must now be considered and the cross Type 1 × Type 18 will be examined first. The results are given in Table VII.

The F_1 was not only taller than Type I (the tall parent) but the inflorescence was more open and the flowers were more sparsely borne on the branches (Plate IX). The F_2 was a mixture of short and tall plants in which tall plants greatly preponderated. The range was the same as the combined range of the two parents and the F_1. By far the largest number of plants had open inflorescences. Compact inflorescences were only to be found on plants approximating to the height of Type 18. All the tall or moderately tall plants had open inflorescences resembling Type 1. Thus a compact inflorescence seems to be incompatible with very long stem internodes. There were, however, short plants with quite open inflorescences (like that of Type I) and plants of which the inflorescences were intermediate in character as well as short, compact plants. There is, apparently, nothing incompatible between an inflorescence with long inflorescence internodes and short stem internodes as the analysis of the existing types had already shown. The following is a graphic representation of the height of the parents, and of the F_1 and F_2 generations in 1912.

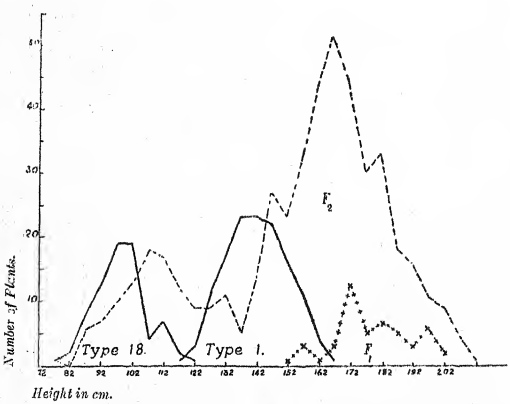


Fig. 1. *Parents. F_1 and F_2 generations of the cross Type 1 × Type 18.*

It will be seen that the form of the curve suggests a 1 to 3 ratio, the break occurring at about 130 cm. This is borne out by the actual numbers 118 : 356 which are practically theoretical. As the parent curves slightly overlap, these numbers cannot be taken as strictly accurate but they do indicate the 1 : 3 ratio. If the short plants only are taken into consideration, it is found

No. 1—tall. No. 60—short, open. No. 61—short, compact.

TYPICAL PLANTS IN THE CROSS F (TYPE 1 × TYPE 18) × TYPE 18

PLATE XIII

that the plants with an open or extended inflorescence form one-quarter of the whole number of short plants (29 in 118).

The F_1 was also crossed back on to both parents. From the union of the F_1 with the tall parent only tall plants with open inflorescences were obtained, the range of variation being approximately equal to the combined range of Type 1 and the F_1. No plants which could not be grouped with these were produced. When crossed back on to Type 18, however, both tall and short plants were produced in approximately equal numbers (58 short to 59 tall), with a distinct break in the series. The inflorescence of all the tall plants was open but it varied from open to compact in the short group. In this group, the plants with an open inflorescence again formed one-quarter of the whole, namely, 14 in 58. In Plate XIII, a tall plant, a short plant with an open inflorescence and a short plant with a compact inflorescence are shown.

The simplest explanation of the above facts would appear to be the following. Group I (tall) differs from group II (short) by a single factor L which affects the length of the internodes both of the stem and of the inflorescence and this character is completely dominant. As, however, short plants with open inflorescences occur, Type 1 must contain an additional factor OO which only affects the inflorescence. In the presence of LL or Ll this factor would not be apparent as all plants containing either LL or Ll would be tall with open inflorescences. In the group of short plants, i.e., those containing LL the effect of the factor OO would be visible and all plants containing OO would have open extended inflorescences. The inflorescences of plants containing oo would be compact. The F_1 OO is apparently intermediate between compact and open with a strong leaning towards the compact form. Further evidence on this point will be given when cross Type 1 × Type 16 is considered.

The results obtained by crossing the tall group of *N. rustica* with the short group (i.e., complete dominance in the F_1 and in the F_2 a 3 : 1 segregation indicating a single factor difference) are similar to those obtained by workers on other crops.[1, 2, 3] Emerson[4] showed that in the case of beans this single factor was connected with the habit of growth. The short beans were determinate in their growth, the tall ones indeterminate.

There is one other point which requires mention in connection with this cross and that is the fact that the break, both in the curve of the F_2 and of the cross Type 18 × F_1 (Type 1 × Type 18), occurs at 130 cm. whereas the limit of

[1] Mendel, G., *Verhandlungen d. Naturf. Ver. Brünn*, IV, 1865.
[2] Bateson, W., and Punnett, R. C., *Report Evol. Com. Roy. Soc.*, 1908.
[3] Keeble, F., and Pellew, C., *Journal of Genetics*, I, 1910, p. 47.
[4] Emerson, R. A., Bull. 7, *Agr. Exp. Sta. of Nebraska*, 1910.

the short parent is about 120 cm. The break is quite distinct in both cases and could not occur anywhere else. Two causes are probably concerned in this (1) the added vigour due to hybridization as shown by the F_1 (2) the increase in height of the short plants which must result from the presence of OO.

The cross Type 1 × Type 16 will now be considered. In general, the results (Table VIII) resemble those of the last cross. The F_1 was taller than Type 1, the tall parent, and had a very open inflorescence. In the F_2, a series of short and tall plants were obtained. All the tall plants had open inflorescences but the short plants were a mixture of plants with open and compact inflorescences. The range of the F_2 was slightly greater than the combined range of both the parents and the F_1, indicating that Type 16 contains a factor not present in Type 1. The second generation does not give so clear an indication of a 3 : 1 ratio probably due to the presence of this additional factor.

No further observations on the relation of tall plants to short were made in this case but the inheritance of the form of the inflorescence was studied among the group of short plants. Five individuals were chosen which approximated to the short parent (Type 16) in height but of which the form of the inflorescence varied. In the F_3, the following results were obtained (the detailed measurements of the height are given in Table VIII and the photographs of the F_2 plants in Plate XIV).

TABLE IX.

Inheritance of the form of the inflorescence.

Plant No.	F_2 generation Form of the inflorescence	No. of plants	F_3 generation Height and form of the inflorescence
A 440	Inflorescence open ..	65	Inflorescences of all are open and like the parent
A 182	Inflorescence open ..	131	Inflorescences of all are open and like the parent plant
A 127	Inflorescence intermediate between open and compact	109	Inflorescences not uniform
A 61	Inflorescence intermediate between open and compact	259	Inflorescences were a mixture of open, compact and intermediate forms resembling the parent plant. Out of a total of 259 plants 62 plants were as compact as Type 16 giving a ratio of 3·2 : 1
A 490	Inflorescence intermediate between open and compact	104	Inflorescences were a mixture as above

PLANTS OF THE F_2 GENERATION OF THE CROSS TYPE 1 × TYPE 16.

Table VIII.

Height Type 1 × Type 16.

	Height of parent	Centimetres 67	72	77	82	87	92	97	102	107	112	117	122	127	132	137	142	147	152	157	162	167	172	177	182	187	192	197	202	207	212	217	222	227	232	237	Total No. of plants	Mean	S. D.	C. of V.
Type 16	..	..	..	..	..	1	3	1	13	7	6	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	34	104·6 ± ·73	6·31 ± ·52	6·03 ± ·49
Type 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	5	2	4	3	4	3	..	1	1	..	..	..	..	..	..	..	24	175·3 ±1·58	11·53 ± ·99	6·57 ± ·57
F_2 Type 1 × Type 16	..	1	..	1	..	1	2	4	8	8	6	12	11	12	11	5	14	9	15	20	18	16	26	22	23	22	29	13	23	11	7	5	3	1	..	3	362	165·4 ± 1·1	32·86 ± ·42	19·87 ± ·25
Type 16	..	..	..	..	2	4	20	22	38	16	5	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	109	99·7 ± ·43	6·78 ± ·31	6·80 ± ·31
Type 1	..	..	..	..	..	..	..	..	..	..	..	3	7	21	33	43	31	31	28	13	9	4	3	..	..	..	..	..	..	..	..	..	..	..	..	..	226	141·7 ± ·52	11·53 ± ·36	8·14 ± ·25
F_1 Type 1 × Type 16	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	2	5	9	13	17	10	12	2	..	..	..	..	..	..	..	..	..	..	..	..	73	167·7 ± ·78	9·90 ± ·55	5·90 ± ·33
F_3 Type 1 × Type 16																																								
440	?	..	..	1	1	4	5	15	12	12	7	8	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	65	102·3 ± ·77	9·27 ± ·59	9·06 ± ·58
490	104	1	4	18	21	25	22	20	18	13	12	5	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	163	93·6 ± ·67	12·72 ± ·47	13·59 ± ·50
182	140	..	..	1	5	7	23	28	28	19	11	7	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	131	100·2 ± ·53	9·01 ± ·37	8·99 ± ·37
61	148	..	..	..	1	3	4	19	38	48	37	41	22	14	13	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	245	111·7 ± ·47	11·04 ± ·33	9·88 ± ·29
127	144	..	..	..	..	1	..	1	8	9	23	17	21	15	10	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	109	118·1 ± ·64	9·94 ± ·44	8·41 ± ·37
Type 16	..	..	..	1	4	10	22	10	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49	91·3 ± ·51	5·33 ± ·36	5·83 ± ·39
Type 1	..	..	..	..	..	..	..	..	..	..	1	1	1	1	..	1	2	1	3	3	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	17	144·7 ±2·58	15·81 ±1·83	10·92 ±1·12
F_4 Type 1 × Type 16																																								
490 – 53	110	2	18	35	39	27	9	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	131	80·9 ± ·38	6·08 ± ·26	7·52 ± ·32
490 –118	91	..	2	5	21	42	36	11	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	119	88·1 ± ·35	5·65 ± ·25	6·41 ± ·28
490 – 60	103	..	6	14	11	26	22	9	10	10	9	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	117	91·2 ± ·67	10·68 ± ·43	11·71 ± ·47
490 – 65	118	..	12	14	23	16	21	16	14	8	6	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	133	97·8 ± ·81	13·86 ± ·55	14·17 ± ·56
490 – 52	128	..	..	..	..	1	2	6	8	19	36	39	10	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	122	111·9 ± ·55	9·06 ± ·39	8·09 ± ·35
440 – 34	?	..	..	..	..	..	5	4	3	13	30	30	26	12	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	125	115·0 ± ·52	8·54 ± ·38	7·54 ± ·33

From the above table it will be seen that in the F_3 the two plants A 440 and A 182 with open inflorescences bred true to an open inflorescence. A 440 was continued into the F_4 when a uniform progeny of 125 plants, all with open inflorescences, was obtained. The plants A 127, A 61 and A 490, in which the inflorescence was of an intermediate character, all gave mixed progenies with open, compact and intermediate inflorescences. In the single case in which a count was made, the plants with compact inflorescences formed about one quarter of the whole.

One plant of the culture A 440 and five plants of culture 490 were grown in the F_4 generation. These five plants included one in which the inflorescence was open, two in which it was compact and two in which it was intermediate in form. The first plant bred true to an open inflorescence. The two compact plants also bred true but differed slightly from one another. A 490–118 resembling Type 16 while A 490–53 was still more compact. The two individuals with intermediate inflorescences gave mixed progenies. These results are summed up in Table X and three of the parent plants are shown in Plate XV.

TABLE X.

Form of the inflorescence in the F_4 generation.

Plant No.	F_3 generation Form of the inflorescence	No. of plants	F_4 generation Height and form of inflorescence
A 440–34	Open	125	Inflorescence all open. Height uniform
A 490–53	Compact	131	Inflorescences all compact. Height uniform and shorter than in Type 16
A 490–118	Compact	119	Inflorescences all compact. Height uniform and equal to Type 16
A 490–56	Open	122	Inflorescences all open. Height uniform and greater than in Type 16
A 490–60	Intermediate	117	Inflorescences a mixture of open, compact and intermediate forms. Height not uniform
A 490–65	Intermediate	133	Inflorescences a mixture of open, compact and intermediate forms. Height not uniform

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tion of A 127. In the F_4 an interesting result was obtained. The two cultures of which the inflorescences were compact, namely, A 490–53 and A 490-118, were grown in long lines next to the parent types. Culture A 490–53 was uniform and decidedly shorter than Type 16. This observation was confirmed by direct measurement (see Table VIII). The average height was found to be 80·9 cm. whereas that of the parent type was 91·3 cm. The low value of the co-efficient of variability points to probable uniformity. Culture A 490–118 was also uniform in height but resembled Type 16. We have thus obtained not only a uniform culture resembling Type 16 but also one about 10 cm. shorter. The inflorescences of both cultures were compact but while that of A 490-118 resembled Type 16 in being slightly open, the inflorescence of A 490-53 was very condensed like that of Type 18. It, therefore, appears probable that Type 16 contains some factor P capable of converting a very compact inflorescence into one slightly more open but yet compact. This factor is absent both from Type 1 and from Type 18. This would agree with the experimental results. In the F_2 of Type 1 (LLOOpp) × Type 16 (llooPP) there would be 25 per cent of short plants, of these one quarter would possess OO and have an open inflorescence while the inflorescences of the rest would all be compact but to varying degrees. Individuals possessing the constitution llOopp and llooPP would breed true but would differ in appearance from one another.

As regards the cultures with open inflorescences, both appeared uniform in the field and taller than Type 16. Table VIII shows that they were probably uniform with an average height of 111·9 cm. and 115·0 cm. respectively, whereas the average height of Type 16 was 91·3 cm. In considering the height of each culture, however, the form of the inflorescence must be considered also. It was noticed that in mixed cultures containing all three forms of inflorescence, the plants with open inflorescences were invariably taller than those with compact inflorescences. The factor, which by lengthening the main stem of the inflorescence causes this to appear open produces an addition in the height of the plant without necessarily affecting the length of the leaf-bearing portion of the stem.

Tall × tall.

The cross tall × tall (Type 1 × Type 5) was not investigated very fully. The F_1 was much taller than either parent and the range of the F_2 was greater than the combined range of both the parents and the F_1. It would thus seem probable that Type 5 contains certain factors which are not present in Type 1. The investigation of this cross appeared somewhat complicated and was

PLATE XV.

F_3 GENERATION OF THE CROSS TYPE 1 × TYPE 16.

not pursued for want of time. Although included in the group of tall types, Type 5 differs markedly both in height and in the form of inflorescence from the other four types. To obtain a simple case of tall × tall, Type 1 should have been crossed with one of the other members of the group.

Many more detailed investigations are necessary to complete the analysis of the factors concerned in the height of the plant and the form of the inflorescence. The above data, however, appear to show the existence (1) of a factor LL which converts a short plant of any kind into a tall plant with an open inflorescence and (2) a factor OO which affects the inflorescence only, converting a compact form into an open one. The existence of two compact forms, differing from one another but yet breeding true indicates the existence of other factors affecting the inflorescence.

IV. Summary.

The experimental results given above appear to justify the following conclusions :—

1. Neither parthenogenesis nor parthenocarpy were observed in the Indian types of *N. rustica*.

2. In the F_1, the value of all the characters (except height) is intermediate between those of the parents. The average height of the plant in the F_1 was greater than that of the tallest parent in all the cases investigated.

3. A frilled leaf margin is dominant to a smooth edge and is caused by the presence of a single factor. A similar result has been recorded for *N. Tabacum*.

4. The relative position of the anthers and stigma and consequently the method of pollination is influenced by factors affecting the length of the pistil and that of the filaments. In the cross—Type 1 (anthers below) × Type 16 (anthers level)—the difference was shown to be due to a single factor which influenced the length of the pistil. In another cross—Type 1 (anthers below) × Type 5 (anthers above)—at least two factors were involved affecting both the length of the pistils and of the filaments.

5. Measurements of the calyx and corolla showed that the F_1 was the exact intermediate between the parents. The F_2 gave a series with a range of variation equal to the combined ranges of the parents. These results are similar to those recorded for *N. Tabacum*.

6. The difference between the tall and the short types of *N. rustica* is due to a single factor L which causes elongation of the internodes of both the stem and the inflorescence. There are probably other factors affecting the

height of the types of the two groups *inter se*. Many of these are connected with the form of the inflorescence. The number of internodes is not significant in the division between tall and short types.

7. The form of the inflorescence is due to factors affecting the length of the internodes of the main axis of the inflorescence and also the proximity of the capsules on the branches. These factors influence the inflorescence only. In two cases, a single factor O was found capable of converting a compact inflorescence like that of Type 18 into an open one. Indications were obtained of the existence of another factor P capable of converting a very compact inflorescence like that of Type 18 into one which is slightly more open such as that of Type 16. It is apparently impossible to obtain a compact inflorescence if LL or Ll is present but both open and compact inflorescences are found in their absence. The presence of OO makes a distinct difference to the height of the plant.

PUSA,
18th August, 1923.

APPENDIX.

Descriptions of the Types used in Hybridization.

Type I. Plants early, tall with long internodes, height 135 cm. *Leaves* inserted at an angle of about 60°, the lamina afterwards curving towards the ground, sub-cordate; apex more acute than in the other types of this group; margin very undulate and curving upwards; surface somewhat puckered; colour dark blue green; average length of petiole 7 cm.; average length of lamina 29·5 cm.; ratio length/breadth 1·13. *Inflorescence leaves* inserted at an angle of about 45°, elliptical to lanceolate; apex acute; margin undulate; surface flat. *Inflorescence* open; the secondary branches long and slender, almost as long as and running somewhat parallel to the main axis. *Flowers* sparse, medium in size; outline straight, no apparent constriction. *Calyx* somewhat dark green, tubular; midrib of sepals well marked; teeth long and pointed. *Corolla* with very distinct lobes; limb never flat or fully expanded; apiculæ conspicuous. *Capsule* medium in size, a little longer than the calyx, round; apex rounded, scarcely umbilicate.

This type is not adapted for self-fertilization. The stamens are shorter than the style throughout the period of development of the flower, and it is only by shaking or by the agency of wind that self-pollination can be effected. If left to themselves, many of the flowers drop and but few capsules are formed. Artificial pollination and crossing with another type were uniformly successful.

Type V. Plants early; tall, height 107 cm. *Leaves* inserted at an angle of 60°; sub-cordate; apex obtuse; margin undulate; surface somewhat puckered; colour blue green; average length of petiole 7 cm.; average length of lamina 24·5 cm.; ratio length/breadth 1·09. *Inflorescence leaves* inserted at an angle of 45°, sub-cordate to ovate; apex obtuse; margin very slightly undulate; surface flat. *Inflorescence* open; secondary branches shorter than the main axis and not parallel to it. *Flowers* somewhat sparse, small in size; outline shows a decided constriction. *Calyx* somewhat globular; midrib of sepals fairly prominent; teeth somewhat acute. *Corolla* with distinctly divided limb, which is quite flat and fully expanded; apiculæ well marked. *Capsule* medium in size, conical, about two-thirds covered by the persistent calyx; apex not umbilicate.

Type V is adapted for self-pollination. When the flower opens, the anthers are well above the stigma and completely cover it, making cross-pollination almost impossible.

Type XV. Plants late, very bushy partly owing to the comparatively large size of the inflorescence leaves; short with short internodes, height 82 cm. *Leaves* inserted at an angle of 45°, sub-cordate; apex obtuse to rounded; margin very undulate; surface very puckered; colour dark blue green; average length of the petiole 9 cm.; average length of lamina 21 cm.; ratio length/breadth ·98. *Inflorescence leaves* inserted at an angle of 30° to 45° and resemble the lower leaves in every way but are smaller. *Inflorescence* neither very compact nor very open; side-shoots long but not as long as the main axis. *Flowers* crowded, slender. *Calyx* tubular; teeth obtuse. *Corolla* with a very slightly divided limb which is flat; apiculæ inconspicuous. *Capsule* small, rounded, almost covered by the persistent calyx; apex blunt and not umbilicate.

In this type self-pollination is predominant, the stamens are raised well above the stigma and completely cover it when the flower opens and the pollen is being shed.

Type XVI. Plants late; dwarf, compact, height 81 cm. *Leaves* inserted at an angle of nearly 90° standing out horizontally from the stem, sub-cordate; apex obtuse; margin flat; surface flat; colour light yellowish green; average length of petiole 7 cm.; average length of lamina 32 cm.; ratio length/breadth 1·19. *Inflorescence leaves* inserted at an angle of 45°, elliptical; apex obtuse; margin entire; surface flat. *Inflorescence* compact but slightly more open than in Types XVII and XVIII. *Flowers* crowded, large. *Calyx* loose and baggy; teeth short and obtuse. *Corolla* with a very slightly divided limb which is flat and fully expanded; apiculæ well marked. *Capsule* medium in size, round, almost covered by the persistent calyx; apex blunt and umbilicate.

In this type both cross and self-pollination are possible, the stamens being approximately equal in length to the style.

Type XVII. Plants very late; dwarf, compact, height 63 cm. *Leaves* inserted at an angle of 45° to 60°, sub-cordate, almost ovate; apex rounded; margin very undulate; surface very puckered; colour somewhat light green; average length of petiole 9 cm.; average length of lamina 34 cm.; ratio length/breadth 1·05. *Inflorescence leaves* inserted at an angle of about 60°, elliptical; apex rounded; margin very undulate; surface very puckered. *Flowers* crowded, large. *Calyx* loose and baggy; teeth short and obtuse.

Corolla with a very slightly divided limb, which is crumpled; apiculæ inconspicuous. *Capsule* large, somewhat conical, two-thirds covered by the persistent calyx; apex rounded and umbilicate.

This type is adapted both for cross and self-pollination, the stamens being approximately equal in length to the style.

Type XVIII. Plants late; dwarf, compact, height 72 cm. *Leaves* inserted at an angle of 45° to 60°, somewhat orbicular; apex obtuse to rounded; margin very undulate; surface very puckered; colour dark blue-green; average length of petiole 9 cm.; average length of lamina 28 cm.; ratio length/breadth 0·97. *Inflorescence leaves* inserted at an angle of 45°, ovate or broadly elliptical; apex obtuse or rounded; margin very undulate; surface very puckered. *Flowers* crowded, large. *Calyx* tubular and loose; teeth short and obtuse. *Corolla* with a slightly divided limb, which is flat and fully expanded; apiculæ inconspicuous. *Capsule* large, round, almost covered by the persistent calyx; apex blunt and umbilicate.

In this type both cross and self-pollination are possible, the stamens being approximately equal in length to the style. This is a vigorous, very large-leaved type and is very like Type XVII, but the leaves are shorter, more orbicular, darker green and thicker.

THE WILT DISEASE OF SAFFLOWER.

BY

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THE safflower (*Carthamus tinctorius*) is grown in many parts of India. In the United Provinces, it is grown as a winter crop largely in the Bulandshahr and Meerut Districts and in small quantities in the eastern districts either as a mixed crop or along the borders.

The increasing economic importance of the crop in India has stimulated its extensive study at all quarters. Howard and Remington[1] have shown its economic value on account of the oil and the dye it yields.

The wilt disease of safflower was for the first time observed at Pusa in the winter of 1920 when the crop was badly affected at the flowering time. Later on it was also found at Sankni in the district of Bulandshahr. The cultivators hold that the disease is of more common occurrence in years of abundant rainfall. This is but to be expected when we consider the life-habits of the causal organism.

The extent of the damage varies according to the character of the season at the time of infection.

The number of plants affected in the fields at Pusa was as high as 30 per cent. in some of the varieties, practically all of which were involved. The disease was brought to notice only when a large number of mature plants began to show sudden wilting; therefore it was not possible to make observations as to how the disease progressed in the field.

Sporadic cases of wilt were known to occur in the safflower crop every year, but no serious notice was taken of it. Although the plots which were badly

[1] Howard, A., and Remington, J. S. *Pusa Agri. Res. Inst. Bull.* 124.

affected were not under safflower in the preceding season, the fungus might have been present in the crop which was grown there the year before. The fungus also attacks varieties of other crops and weeds and through them it can be carried from year to year; at the same time the infective material in the form of sclerotia is increased considerably which causes the disease to spread over increasingly wider areas.

It is difficult to formulate the exact conditions which led to a severe attack at Pusa in 1919-20. Ploughing in of the stubbles of the previous crop might have increased the infective material considerably. The favourable temperatures of between 50°–60°F. in January and February combined with high humidity accompanying a rainfall of 1·7″ in February and 1·11″ in March favoured the growth of the fungus and resulted in the severe damage observed. The earliest cases of wilt were noticed in a few plants towards the end of January.

Symptoms.

The wilted plants have dense white growth of mycelium at their bases. Large black sclerotia are produced just below the soil level, on the surface of the crown and roots. These are very loosely attached so that the plants have to be carefully pulled up to get the sclerotia in position. The sclerotia are also produced inside the stem (Pl. I, fig. 5). In the diseased crop at Pusa an apparently healthy grown up plant showed the next day a little yellowing of the leaves and very quickly the whole plant dried up. The cortical tissue in the lower part of the stem came off into shreds. A characteristic feature of the diseased plant at an advanced stage was the ease with which the flower-heads broke off from their stalks, leaving behind an outer involucre of bracts. In a healthy plant, on the other hand, the flower-heads are firmly attached to the stalks and cannot easily be separated. This condition was due to the development of a large black pear-shaped sclerotium in the thalamus of the flower (Pl. III, fig. 3) and the thalamus changing into a powdery mass.

The attacked plants in some cases resisted wilting and produced apparently normal fruits. These fruits, however, at maturity, were found to contain either no seeds or only defective seeds.

Cultural characters.

The mycelium is of the typical *Rhizoctonia* form showing the usual constriction and septation in branching (Pl. I, fig. 1). The cells in young culture show a great variation in their size. They are from 115μ to 190μ in length by 16μ to 21μ in breadth. The first septum is usually formed at a distance of 20μ to 47μ from the parent hypha.

The sclerotia are very irregular in shape (Pl. I, fig. 5), varying from roundish to elongate. They are thick solid bodies about 2-12 mm. long. Those formed at the bases of flower-heads have in majority of cases a curious pear-shaped appearance, after the shape of the conical thalamus (Pl. III, fig. 1). The sclerotia begin as white hard spots in the mycelium; the outer surfaces afterwards turn black. Drops of a clear shining liquid are very prominent on the developing sclerotia and can be seen even after they have blackened. The sclerotia are formed on the surface of the mycelium from which they can be easily detached. Usually they have a covering of a semi-persistent thin mycelial membrane. Their outer surface is somewhat rough. The interior is formed by not very compactly interwoven hyphæ and thus when placed in water they float for a time. The outer two or three layers of the sclerotia appear brownish black while the interior is white (Pl. I, fig. 6). Often the recently formed sclerotia, in artificial cultures, show a very faint tinge of pink inside.

Peculiar tufty branchings (Pl. I, figs. 3, 4) are frequently formed in cultures of the fungus especially in glucose agar medium. These are seen at the edges of the slant cultures where the mycelium comes in contact with the surface of the glass. These branches are comparable to the branchings of *Botrytis* and *Sclerotinia* described by Smith[1] and may similarly be called the organs of attachment or appressoria.

Perfect apothecial stage has not been obtained so far. The sclerotia placed in moist sand and sawdust did not produce any apothecia. In a few cases, however, where they were sown on ordinary moist earth in a large petri dish the sclerotia gave out brownish outgrowths, about 4-10 mm. long, which developed distinct apothecial cups at their tips (Pl. I, figs. 2, 7), but inspite of every care they shrivelled up before any asci were produced. In cultures on corn, oat, wheat and bean-meal agar, there was sometimes development, from the surface of the sclerotia, of slender stalks either singly or in large numbers measuring as much as 2 cm. in length (Pl. II, fig. 4; Pl. III, fig. 2). They remained sterile till they dried up. Light appears to have little effect upon the development of these stalks.

No apothecial outgrowths could be found even with frequent and careful search in the fields, throughout the year.

A distinct spore form occurs in cultures 9 or 10 days old. The fertile hyphæ bear conidiophores as simple branches which may repeatedly branch to form a cluster of sterigmata (Pl. II, figs. 1, 2). These bear conidia at their

[1] Smith, R. E. *Botrytis* and *Sclerotinia*; their relation to certain plant diseases and to each other. *Bot. Gaz.*, p. 29, 1900.

tips in chains. The conidia are from 3-3·5μ in diameter, hyaline, with usually a highly refractive granule inside. Such spore form is always abundantly produced in bean, corn, wheat, oatmeal media, while the glucose agar medium although showing luxuriant growth of mycelium does not seem very suitable for it. The fungus does not grow well on safflower seed medium. These conidia could not be made to germinate. They failed to germinate in water, 1 per cent. glycerine, 1 per cent. cane sugar, turnip juice, horsedung solution, cowdung solution, and safflower leaf juice either at the ordinary winter temperatures of 20°–22°C. or at the higher temperature of 37°C. Efforts on glucose agar medium were equally unsuccessful.

Pathogenicity.

Germinating seedlings inoculated with bits of hyphæ from a pure culture rotted very quickly, forming copious mycelial growth on the surface which in time produced sclerotia. Young plants inoculated with the fungus at the soil level quickly succumbed and the fungus spread through the soil to the neighbouring healthy plants and soon killed them. Large plants with hard tissues are not killed immediately. The spread of the mycelium within the tissues of the host depends upon the moisture condition of the soil and atmosphere. That from the infected spots was completely checked when the plant was placed in dry open air. In all these cases the fungus could easily be recovered from infected plants in pure culture.

The mycelium grows in the tender tissues of the leaf and stem (Pl. II, fig. 3) and causes disorganization of the cells, changing them into soft pulpy mass. It penetrates the inner tissues of the stem and also grows in the pith where it forms large black sclerotia. The young plants so attacked quickly die while the maturer plants withstand wilting for some time.

It can be gathered from the above that if the conditions are favourable for the growth of the fungus when the plants are very young, a wholesale destruction of the crop may not be an improbability. Nevertheless, depending upon the time of infection and the rapidity of the spread of the disease in the field we meet with wilting of plants at different stages of their growth.

Hosts of the fungus.

A few other plants in the field besides safflower were also found infected with this fungus. Inoculations were, therefore, made on some of the more important winter crops and weeds. Wheat, oat, gram, mustard, pea, and potato, all took infection readily. Of the farm weeds the most common *Chenopodium album* (vernacular *bathua*) and *Asphodelus tenuifolia* (vernacular

pyazi) were very highly susceptible, while *Argemone* was less so and *Melilotus indica* did not take the infection at all.

Longevity of the sclerotia.

The sclerotia of the fungus do not seem to live very long. Those produced in the culture tubes between October and December 1920 showed very little germination in November 1921, and practically all in the tubes rotted away by October 1922. Only 14 per cent. of the sclerotia were found germinating after one year. Sclerotia which were gathered from the host plants in March 1920 grew in November 1920, but no germination was obtained in November 1921 or in 1922 and 1923 although they were still apparently sound.

Systematic.

The morphological characters of the safflower wilt fungus are exactly alike those of the large sclerotia type of *Sclerotinia Libertiana* Fuckl. described by Smith.[1] The sclerotia of this fungus although do not fruit easily, in rare cases have been found to produce distinct apothecial cups. A wilt of sunflower very closely resembling in effects that of safflower has been described by Bisby[2] in Manitoba and by Morris and Swingle[3] in Montana. The latter workers also found the sclerotia of their fungus which they identified as *S. Libertiana* fruiting with difficulty. A stalk disease of potato which is also similar to this wilt of safflower has been described by Pethybridge.[4] This latter he holds to be due to *Sclerotinia sclerotiorum* which according to him was "then known as *S. Libertiana* Fuckl., but known at an earlier date as *Peziza sclerotiorum* Lib., and now in accordance with the very widely accepted international rules of botanical nomenclature more correctly called *Sclerotinia sclerotiorum* Mass." This fungus, therefore, which causes the safflower wilt may be called *Sclerotinia sclerotiorum* Mass.

The spore form of the fungus described before may, however, give rise to certain doubt regarding its probable relationship with *Botrytis*. Brierley[5] in his recent studies on *Botrytis cinerea* noticed a microconidial stage associated with the normal type of conidia. This microconidial stage may be comparable

[1] Smith, R. E. *Loc. cit.*

[2] Bisby, G. R. *Sclerotinia* disease of sunflower in Manitoba. Abstracts in *Phytopathology*, XI, No. 1.

[3] Morris, H. E., and Swingle, D. B. An important new disease of cultivated sunflower. Abstracts in *Phytopathology*, XI, No. 1.

[4] Pethybridge, G. H. Investigations on potato diseases. *Jour. Dept. Agri. and Tech. Inst. Ireland*, XVI, No. 4.

[5] Brierley. The microconidia of *Botrytis cinerea*. *Kew Roy. Bot. Gardens Misc. Bull.* 4, 1918.

to the conidial stage of this fungus. From his own investigations and those of many others Brierley is of opinion that certain factors can drastically alter the normal life-cycle of *B. cinerea* and that any spore stage may be reversed or eliminated. But unlike Brierley as in his investigations, the writer has neither been able to get the conidia to germinate nor could he find out the circumstances under which a normal *Botrytis* stage, if any, may be formed in the cultures of safflower wilt fungus.

Some species of *Sclerotinia* described by De Bary[1] and Gilbert and Bennett[2] are known to produce similar spores either on the apothecia by the germination of ascospores or on the vegetative mycelium, which also did not germinate. As mentioned before, the conidia of this fungus cannot be germinated and can be regarded as functionless. It is indicated, therefore, that a *Botrytis* stage is absent from the life-cycle of this fungus.

Pethybridge[3] has given the authority of all workers to show that *Botrytis* stage is absent in *S. sclerotiorum* and that there is no connection between the two.

A distinguishing character of *S. sclerotiorum*, according to Tubeuf and Smith,[4] is the conical funnel-shaped depression in the hymenial disc. This depression which is clearly seen in this fungus (Plate I, fig. 7). Hence it is concluded that the fungus under study is *Sclerotinia sclerotiorum* Mass.

A comparison of *Rhizoctonia Napi* West. of Shaw and Ajrekar[5] with the safflower organism suggests their complete identity. Their morphological and anatomical characters are very much alike. As in safflower fungus, the faintly pink tinge of the interior of the sclerotia has been noticed in their sclerotia as well, which they have described as flesh-coloured (although in one place they describe it as white in the interior). The spore forms are similar. The range of host plants and the symptoms are almost the same. There is, therefore, every reason to believe that the *Rhizoctonia Napi* West. of these authors is really *Sclerotinia sclerotiorum* Mass.

A comparison of the description of these authors has led Pethybridge[6] also to suggest that it is clearly a species of *Sclerotinia*.

Sclerotinia sclerotiorum which is then of considerable economic importance in India may be regarded as a new addition to the few species of *Sclerotinia* so far recorded in this country.

[1] De Bary. *Comparative morphology and biology of fungi, mycetozoa and bacteria.*

[2] Gilbert, A. H., and Bennett, C. W. *Sclerotinia Trifoliorum*, the cause of stem rot of clover and alfalfa. *Phytopathology*, VII, No. 6.

[3] Pethybridge, G. H. *Loc. cit.*

[4] Tubeuf and Smith. *Diseases of plants induced by cryptogamic parasites*, p. 265.

[5] Shaw, F. J. F., and Ajrekar, S. L. The genus *Rhizoctonia* in India. *Mem. Dept. Agrs. India, Bct. Ser.*, VII, No. 4.

[6] Pethybridge, G. H. *Loc cit.*

Control measures.

The temperatures which the sclerotia are able to withstand under diverse conditions are very difficult to determine with accuracy. The results of the immersion into water for 5 minutes are given in the table below.

Number of sclerotia	Temperature at which immersed	Number germinating	Remarks.
	°C.		
10	98·8	nil	
10	80·0	,,	
10	70·0	,,	
10	60·0	,,	
10	50·0	,,	
10	40·0	9	The controls germinated in each case.
10	45·0	9	
25	46·0	18	
25	47·0	3	
25	48·0	1	
25	50·0	nil	
25	51·0	,,	

The sclerotia were treated with corrosive sublimate solution, 1 in 1000, for about 15 minutes and washed with sterile water before immersion. They were afterwards planted in agar slants. The controls germinated in each case.

It will be seen from the above table that a temperature of between 48°—50°C. approaches the thermal death point of the fungus. It is not, however, likely that the sclerotia will be killed by the summer soil temperatures which may not ordinarily be more than 40°C. at a few inches below the surface. Neither does there appear any chance of starving out the fungus with such a large number of host plants by a practice of long rotation. Any treatment of the soil by sterilization also is out of question from the point of view of expenses.

The fungus does not survive in the seed obtained from the diseased crop as such seed gave a clean crop in the following season.

Taubenhaus[1] found that infection with *Sclerotium Rolfsii* was not possible if the mycelium or sclerotia were buried more than 5″ deep and successful inoculation was obtained only when the fungus was not covered more than ½″–1″ deep. The writer's experience has been in the case of potato *Rhizoctonia* (identified by Shaw and Ajrekar as *Rhizoctonia destruens*) that the growth of

[1] Taubenhaus, J. J. Recent studies on *Sclerotium Rolfsii* Sacc. *Jour. Agri. Res.*, XVIII, 1919.

mycelium and the infection of tubers could be met with at about 5″ or 6″ below the surface provided the moisture is present and the temperature is suitable.

Smith[1] observed that sterilizing 3″ of the surface soil checked the disease due to *S. Libertiana* even in the most infected beds. It can be inferred from the above statement that either the sclerotia do not happen to pass down deeper than 3″ or that they do not germinate below that depth. A reasonable explanation seems to be that those sclerotia that remained in the upper 3″ might have been killed by sterilization while those below that depth failed to germinate. Following this argument as well as from the writer's experience it appears that burying the sclerotia very deep might considerably reduce infection.

The most practicable directions of control, therefore, are, firstly, the careful collection and destruction of infected plant material and, secondly, a very deep ploughing to secure the burial of any remaining sclerotia to a depth of more than 6″. Careful and clean weeding can prevent the rapid spread of the disease in the field.

Summary.

1. Low temperature and abundant moisture are very favourable to the growth of the fungus causing safflower wilt. The fungus grows on many different media.

2. The sclerotia do not seem to live longer than a little over one year.

3. The causal organism is *Sclerotinia sclerotiorum* Mass. It is identical with *Rhizoctonia Napi* West. of Shaw and Ajrekar, which should correctly be identified as such.

4. The sclerotia are killed by immersion in water at 50°C. for five minutes.

5. The practicable control measures are :—

(*a*) Collection and destruction of infective material.

(*b*) Deep ploughing.

(*c*) Clean weeding.

The writer is thankful to Dr. E. J. Butler, Director, Imperial Bureau of Mycology, London, for suggestion of the subject of investigation while working at Pusa, and desires to acknowledge his indebtedness to Mr. P. K. Dey, Plant Pathologist to Government, United Provinces, Cawnpore, for ever ready help and criticisms.

[1] Smith, R. E. *Loc. cit.*

EXPLANATION OF PLATE I.

Fig. 1. Mycelium of Actinomyces scleroticus Mass. from a culture. (× 225)
" 2. A sclerotium showing superficial outgrowths. (× 5)
Figs. 3, 4. Forms of organs of attachment. (× 252)
Fig. 5. Stem of a culture showing septate hyphæ. (natural size)
" 6. Section of sclerotium. (× 450)
" 7. Section of an aborted cell. (× 103)

Explanation of Plate I.

Fig. 1. Mycelium of *Sclerotinia sclerotiorum* Mass, from safflower. (× 553)

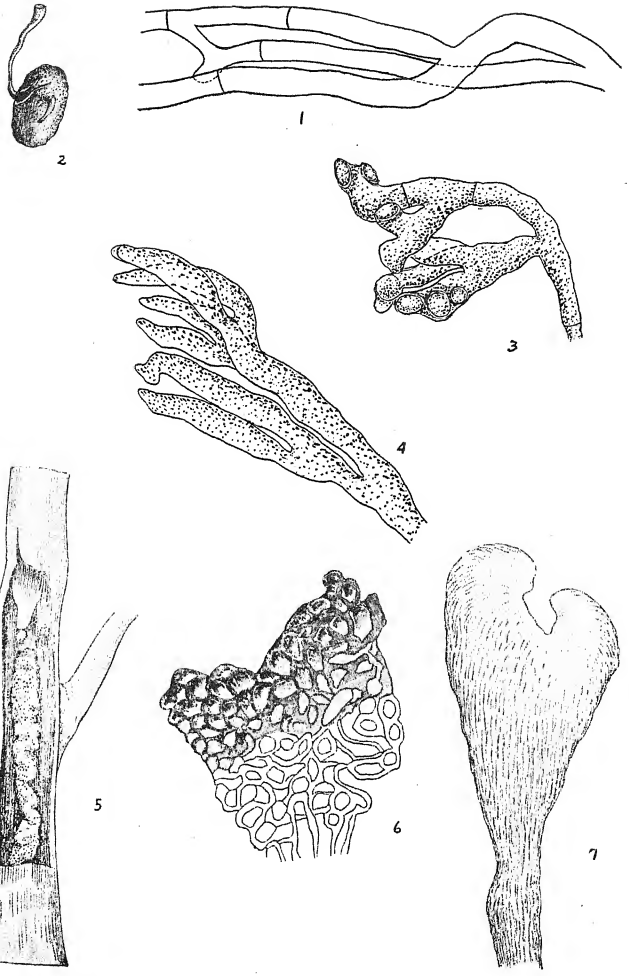
„ 2. A sclerotium showing apothecial outgrowths. (× 2)

Figs. 3, 4. Forms of organs of attachment. (× 535)

Fig. 5. Stem of safflower showing sclerotia in the pith. (natural size)

„ 6. Section of sclerotium. (× 480)

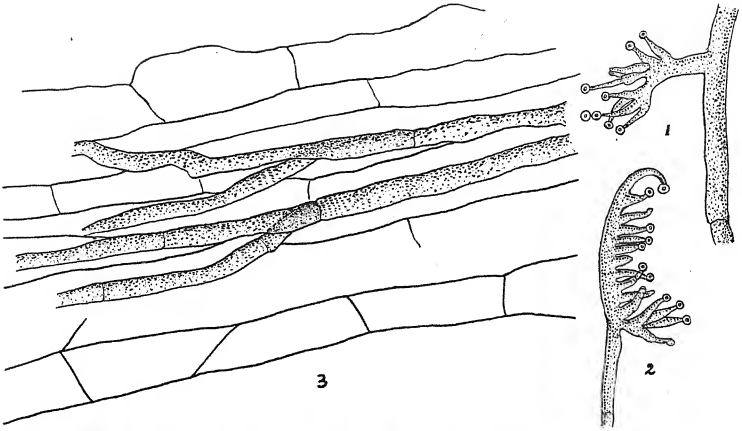
„ 7. Section of an apothecial cup. (× 103)

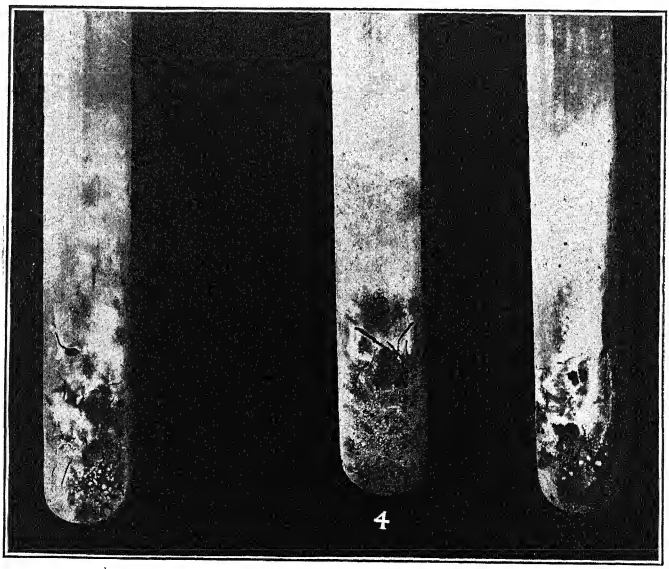


SCLEROTINIA SCLEROTIORUM.

Explanation of Plate II.

Figs. 1, 2. Conidiophores with conidia, from culture. (× 553)
Fig. 3. Hyphæ in the tissue of the host. (× 480)
„ 4. Sclerotia in culture tubes showing slender outgrowths.





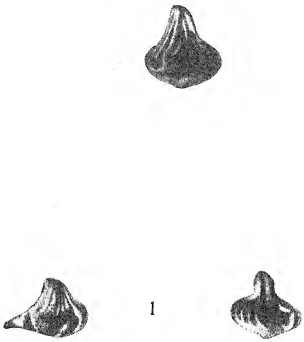
SCLEROTINIA SCLEROTIORUM.

Explanation of Plate III.

Fig. 1. Pear-shaped sclerotia from the flower of safflower.

„ 2. Sclerotia showing the development of numerous slender stalks.

„ 3. Flower-head detached from the thalamus showing the sclerotium inside. (½ natural size.)



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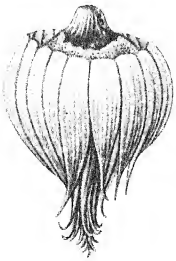

3

SCLEROTINIA SCLEROTIORUM

CONTENTS.

[illegible]

STUDIES IN INDIAN FIBRE PLANTS.

No. 3. ON THE INHERITANCE OF CHARACTERS IN *HIBISCUS SABDARIFFA* L.

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I. INTRODUCTION.

Roselle (*Hibiscus Sabdariffa* L.), known in the vernacular as *mesta, patwa, lal ambari* or *kempu*, is grown for a variety of purposes in many parts of India, Ceylon, and the West Indies. Almost every part of the plant can be utilized. A strong silky fibre—Roselle hemp—is obtained from the stems; the fleshy calyces and leaves are used for food, while the seeds are employed in medicine. The study of this species was begun at Pusa some years ago when four varieties were isolated and described, namely—*ruber, albus, intermedius* and *Bhagalpuriensis*.[1] These four varieties are almost identical morphologically. In var. *Bhagalpuriensis* the calyx is slightly more twisted and obtuse than in the other three varieties. This is the only difference in form, which could be detected. With this minor exception, the sole differences between the varieties lie in the distribution of colour.

Roselle is admirably adapted for the training of students in the technique of plant-breeding. The cultivation is simple, the plants are robust, practically immune to pests and not at all sensitive to water-logging or to defective soil

[1] Howard, A., and Howard, G. L. C. Studies in Indian Fibre Plants, No. 2.—On some new varieties of *Hibiscus cannabinus* L., and *Hibiscus Sabdariffa* L., *Memoirs of the Dept. of Agr. in India* (*Botanical Series*), IV, 1911, p. 35.

aeration. The flowers are large enough for easy manipulation but the experimental plants require constant attention as the flowers open singly. Moreover, this species flowers in November and December between the monsoon and cold season crops when other material suitable for study is scarce. The uniformity in the form and size of the plants enables the student to concentrate on the colour differences. In order to employ Roselle to the best advantage for training purposes, a thorough study of the colour factors was first of all necessary. An investigation into the chemical and physiological factors responsible for these colour differences was also contemplated. Unfortunately, however, the plants require a great deal of space. It is impossible to grow them closer than three feet apart each way. Owing to the expansion, during recent years, of the work on other crops, the pressure on the limited area of land at the disposal of the Botanical Section has been so great that only a very small space could be spared for the Roselle cultures. This circumstance has delayed the work considerably. As the continuation of the investigation is doubtful, we have considered it advisable to publish the results so far obtained. Some of the characters have been worked out in detail, others require further study.

The following general description of the four varieties employed will help to bring out the various differences in the distribution of the colour factors :—

Var. *ruber* (PLATE I).

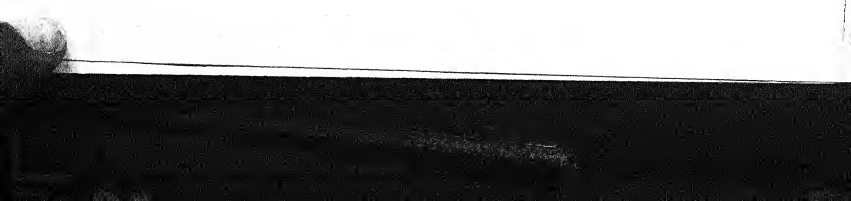
Stem dark red. *Stipules* dark red. *Leaves* green with some red colour on the lower surface of the veins, sometimes also on the upper surface; gland on the midrib colourless; petiole dark red except for a narrow strip on the under surface; pulvinus red. *Peduncle* red. *Epicalyx* red. *Sepals* red; central gland on mid-nerve greenish. *Corolla* yellow, with a deep crimson eye, turning a deep salmon pink on withering. *Stamens* staminal tube red; pollen and anthers deep red. *Stigmas* red. *Seedlings* stem green with some red below the cotyledonary leaves; petiole red on the upper surface, green below; leaves green.

Var. *albus* (PLATE II).

Stem green. *Stipules* green. *Leaves* green, no red colour on the veins; pulvinus green; petiole green. *Peduncle* green. *Epicalyx* green. *Sepals* yellowish green; apices yellow when ripe. *Corolla* yellow with colourless eye. *Stamens* staminal tube white showing up the yellow pollen. *Stigmas* white. *Seedlings* stem, petiole and cotyledonary leaves green.

HIBISCUS SABDARIFFA, L. var. RUBER.

HIBISCUS SABDARIFFA. L. var. ALBUS.



HIBISCUS SABDARIFFA. L. var. INTERMEDIUS.

HIBISCUS SABDARIFFA, L. var. BHAGALPURIENSIS.

Var. *intermedius* (PLATE III).

Stem green with some diffused reddish colour and with deep red triangular patches both in the axil of the leaves and also immediately below the petiole. *Stipules* green. *Leaves* green with some reddening on the upper surface of the veins; pulvinus red. *Peduncle* green. *Epicalyx* green. *Sepals* yellowish green; apices green when ripe, a red spot in the central gland on the mid-nerve. *Corolla* yellow with a deep crimson eye, remaining yellow on withering. *Stamens* staminal tube red; pollen deep orange. *Stigmas* red. *Seedlings* indistinguishable from those of *ruber*.

Var. *Bhagalpuriensis* (PLATE IV).

Stem green with some diffused red colour and a deep red triangular patch in the axil of the leaves. *Stipules* green. *Leaves* green, veins green; petiole green with a certain amount of diffused redness, pulvinus green. *Peduncle* green. *Epicalyx* green. *Sepal* bright green with splashes of red; central gland with a red spot. *Corolla* yellow with a crimson eye which is less deeply crimson than in *intermedius*, salmon pink when faded. *Stamens* staminal tube red; pollen less deeply orange than in *ruber* and *intermedius*. *Stigmas* red.

All the four varieties are normally self-fertilized and set well under bag. Pollination is, however, occasionally effected by humming birds but this occurs so rarely that natural cross-fertilization can be ignored in practice. As a precautionary measure, the flowers of all the plants used in these investigations with very few exceptions, were protected. Two minor difficulties were encountered in the work, namely, (1) the hardness of the seed coat in *Bhagalpuriensis* and in the hybrids derived therefrom and (2) the dormancy of the seeds left in the soil. The first difficulty was overcome by pricking before sowing the seeds of *Bhagalpuriensis* and of the hybrid cultures in which this variety was one of the parents. This, although very laborious when the number of cultures was large was uniformly successful. The second difficulty can only be partially avoided by a long rotation and by germinating the fallen seeds on the surface of the ground before they are buried by the plough.

II. THE EXPERIMENTAL RESULTS.

The four varieties were all crossed *inter se*, *i.e.*, *intermedius* × *Bhagalpuriensis*, *intermedius* × *ruber*, *intermedius* × *albus*, *Bhagalpuriensis* × *ruber*, *Bhagalpuriensis* × *albus* and *ruber* × *albus*. In addition, a certain number of crosses

were made between the four varieties and some of the new extracted forms. The F_1 generations were always uniform and the reciprocals alike.

1. The presence of red or crimson colour.

As regards the presence of red colour, the four varieties and the homozygotic phenotypes derived from them by hybridization can be divided into three main groups: (1) those in which the general colour of the plant is green with a certain amount of red distributed on various parts in definite patches: these patches may be accompanied, as in *intermedius* and *Bhagalpuriensis*, by very light general flushes—pink or brown—the tone of which is not sufficiently deep to obliterate them, (2) those like *ruber* in which all such markings are covered by a general crimson colouration of the plant, (3) those like *albus* in which no red or crimson colour at all is present.

The first subject investigated was the nature of *albus*. The general green appearance of this variety might be due either to the absence of all the factors responsible for colour in the other varieties—in which case it would be of the greatest use as an analyser—or the colour factors might be present in a suppressed state. In the investigation of the genetic constitution of *albus*, the first cross to be considered is that of *ruber* × *albus*. The general stem colour of *ruber* is crimson. This colour extends over most of the other parts of the plant but the extreme base of the stem is green. This point proved to be of importance in distinguishing true *ruber* from other crimson forms produced by hybridization. The leaves of *ruber* are green with some crimson on the lower surface of the veins. The petiole is crimson except for a narrow strip on the under surface; the peduncle, epicalyx and sepals are all crimson. The staminal tube, pollen, anthers and stigma are also crimson. *Albus* is very different. There is no red colour on any portion of the plant. The general tone is a yellowish green, quite different from the brighter green of *intermedius*. The apices of the sepals are yellow when mature. The staminal tube and stigmas are white, the pollen yellow.

The F_1 between *ruber* and *albus* was crimson and was practically identical with the *ruber* parent. In the F_2 (Table I), a mixture of crimson and green plants was obtained in which the crimson plants resembled *ruber* and the green *albus*. Nothing in any way different from the two parents was produced.

TABLE I.

The F_2 generation of the cross ruber × albus.

No. of culture		Total No. of plants	Crimson like *ruber*	Green like *albus*	Ratio crimson : green
Ruber × albus	1	300	235	65	..
	2	298	232	66	..
	3	313	235	78	..
	4	505	370	135	..
	5	298	236	62	..
	6	225	180	45	..
Albus × ruber	7	199	154	45	..
	8	230	178	52	..
	9	298	236	62	..
TOTAL		2,666	2,056	610	3·4 : 1
Expectation		....	*1999·5*	*666·5*	....

TABLE II.

The F_3 generation of the cross ruber × albus.

No. of culture	Colour of parent	F_3		
		Total No. of plants	Crimson	Green
13	green	42	....	42
14	Do.	55	....	55
15	Do.	66	....	66
16	Do.	45	....	45
17	Do.	5	....	5
19	Do.	46	....	46
20	Do.	28	....	28
18	Do.	29	1*	28
TOTAL	....	316	1	315
Expectation	....	....	*0*	*316*

* Probably a natural cross as in this case the flowers of the parent were not protected.

TABLE II—*contd.*

No. of culture	Colour of parent	F_2		
		Total No. of plant	Crimson	Green
1	crimson	40	40	....
6	Do.	38	38	....
11	Do.	11	11	....
TOTAL		89	89	0
Expectation		....	*89*	*0*
2	crimson	41	22	19
3	Do.	53	39	14
4	Do.	41	29	12
5	Do.	89	66	23
7	Do.	35	25	10
8	Do.	52	44	8
9	Do.	77	50	27
10	Do.	77	58	19
12	Do.	25	18	7
TOTAL		490	351	139
Expectation		....	*367·5*	*122·5*

The experimental numbers obtained do not agree very closely with expectation but the error is reversed in the two generations.

Similar results were obtained when *albus* was crossed with the two varieties which possess green stems with red markings, *intermedius* and *Bhagalpuriensis*. The F_1 in both cases was predominantly crimson and very like *ruber* although certain differences in detail could be detected. In the F_2,

the following types were found :—(1) green plants without any red at all, (2) green plants with red markings and (3) plants predominantly crimson. The following are the numerical results obtained :—

TABLE III.

The F_2 generation of the crosses intermedius × albus and Bhagalpuriensis × albus.

Cross		Total No. of plants	Plants either crimson or with red markings	Plants entirely green	Ratio crimson : green
Intermedius × albus	A	45	37	8	..
	B	76	58	18	..
	C	85	70	15	..
	D	37	27	10	..
	E	84	59	25	..
	F	66	43	23	..
TOTAL		393	294	99	3 : 1
Expectation		..	*294·5*	*98·5*	
Albus × Bhagalpuriensis	A	82	59	23	..
	B	73	58	15	..
	C	86	59	27	..
	D	78	64	14	..
	E	79	69	10	..
	F	80	59	21	..
Bhagalpuriensis × albus	G	78	54	24	..
	H	74	55	19	..
	I	70	51	19	..
	J	61	44	17	..
	K	95	73	22	..
	L	86	59	27	..
TOTAL		942	704	238	3 : 1
Expectation		..	*706·5*	*235·5*	

Certain of the crimson plants and some of the green plants with red markings again split in the following generation into plants with colour and green plants, the green plants forming approximately one quarter of the whole. The results are given in the following Tables :—

TABLE IV.

Crimson plants giving crimson and green plants in the F_3 generation of the cross Bhagalpuriensis × albus.

No. of culture	Crimson plants	Green plants	Ratio crimson : green
A 6	22	5	
A 7	46	22	
A 23	11	5	
A 25	36	11	
A 30	58	15	
A 40	52	17	
A 42	25	6	
A 43	54	14	
A 55	49	25	
A 61	35	12	
A 66a	58	20	
TOTAL	446	152	2·9 : 1
Expectation	*448·5*	*149·5*	

TABLE V.

Green plants with red markings giving a certain number of entirely green plants in the F_3 of the cross Bhagalpuriensis × albus.

No. of culture	Green plants with red markings	Green plants	Ratio crimson : green
A 3	42	15	
A 14	40	19	
A 12	40	18	
A 15	34	17	
A 63	48	21	
A 64	43	18	
A 59	58	16	
A 71	32	5	
A 80	60	20	
A 75	50	16	
A 76	40	22	
TOTAL	487	187	2·6 : 1
Expectation	*505·5*	*168·5*	

Similar results were obtained in the F_3 of *intermedius* × *albus*. In all the crosses, plants which are entirely green bred true to this character in the succeeding generations. These results point to the absence of a colour producing factor or the presence of a colour inhibiting factor in *albus*. Taking into consideration the relation between anthocyanin and flavone found by many investigators and the fact that the whole appearance of *albus* is yellowish green rather than green, it seems probable that this variety contains the yellow flavone. *Ruber* contains a factor R which converts this into anthocyan. The green plants extracted in the F_3 from the green plants with red markings in the crosses of *albus* with *intermedius* and *Bhagalpuriensis* do not possess the factor for the crimson colour of *ruber* and are therefore green and not yellowish green. These extracted green plants when crossed among themselves or with *albus* gave in the F_1 and succeeding generations green plants only, no coloured plants of any kind being produced. When crossed on to *Bhagalpuriensis* they gave a mixed progeny in the F_2—plants with red markings and plants entirely green. In most cases, the coloured portion of the cultures consisted of individuals with very varied markings on the stems and leaves but in no case were the markings less than those of *Bhagalpuriensis*. When crossed with *ruber*, a mixed progeny of red plants, of green plants with red markings and of entirely green plants were produced in the F_2. It is clear that the extracted greens were merely green plants with red markings in which these markings cannot find expression owing to the absence of the colour producing factor R. In no case has it been possible either to obtain any green plant without X (the factor or group of factors responsible for the markings of *Bhagalpuriensis*) or to break this down into several factors.

2. The red patches on the veins, petioles and stems.

The red or brown colouring of the stem is present in two forms—either as well defined patches of dark red or as flushes or washes with indeterminate outlines which occur over large portions of the stem. The flushes vary in colour and distribution. As this investigation showed that there was no genetic connection between these two systems of colouring, they will be treated separately.

The only two varieties in which the distribution of the isolated patches of colour could be studied were *intermedius* and *Bhagalpuriensis*. The following Table and Plate V show the differences between the two varieties in respect to these characters.

TABLE VI.

Differences in the stem and leaf markings of intermedius and Bhagalpuriensis.

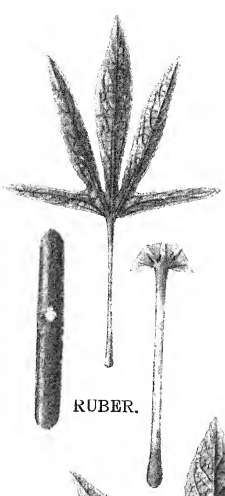
	Intermedius	*Bhagalpuriensis*
Stem	Green with two triangular red patches, one in the leaf axil and one below the petiole	Green with one triangular red patch in the axil of the leaf
Pulvinus	Deep red	Green
Leaf veins	A red spot on all the five primary veins	A red spot on the four outer primary veins only, the central one green

Bhagalpuriensis represents what may be termed the basic form as regard the colouration of the veins, petioles and stems. It has not been found possible to produce by hybridization any plant without the factors for these markings. It is true that perfectly green phenotypes were obtained but, as in the case of *albus*, these proved to be merely potentially coloured forms in which the coloured markings were suppressed. The red triangular patch on the stem in the axil of the leaf is apparently inseparable from the four dots on the veins.

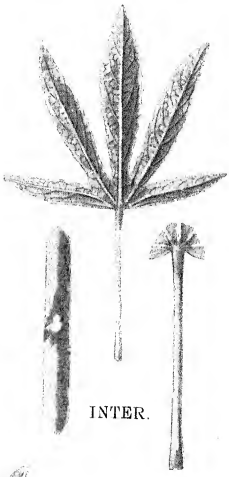
Intermedius × *Bhagalpuriensis.*

In all the characters investigated, the F_1 was uniform and intermediate between the two parents. The seeds of five F_1 plants were sown separately in 1912 and the F_2 in each case consisted of a series of types representing every conceivable gradation between the parents but with nothing greater or less than these. A definite statistical examination of the F_2 proved to be impossible for the following reasons. In the first place, there is a certain amount of variation due to environment and age. As the leaves, stems and petioles grow older they become red all over. Sunlight and shade also effect the intensity and extent of the colour, intense sunlight producing the effect of age. In the second place, the large number of factors involved in so small a phenotypic difference made identification of the various genotypes very difficult. During the course of these investigations, it was found that no less than three factors were concerned in building up the colour markings of *intermedius* from those of *Bhagalpuriensis*. The eight homozygotic phenotypes, resulting from the

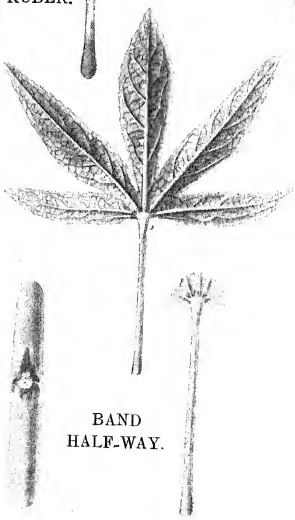

ALABAMA AGRICULTURAL EXPERIMENT STATION
LIBRARY.



RUBER.



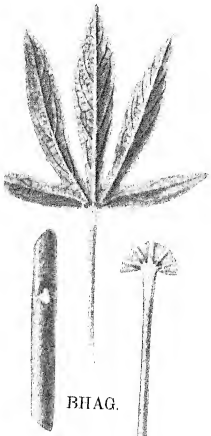
INTER.



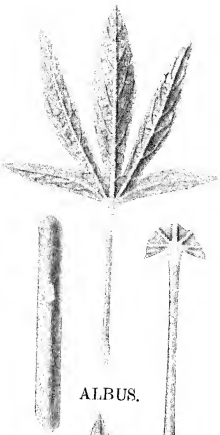
BAND
HALF-WAY.

WA

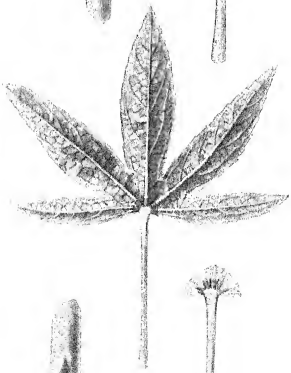
RED PATCHES ON THE VEINS,



BHAG.



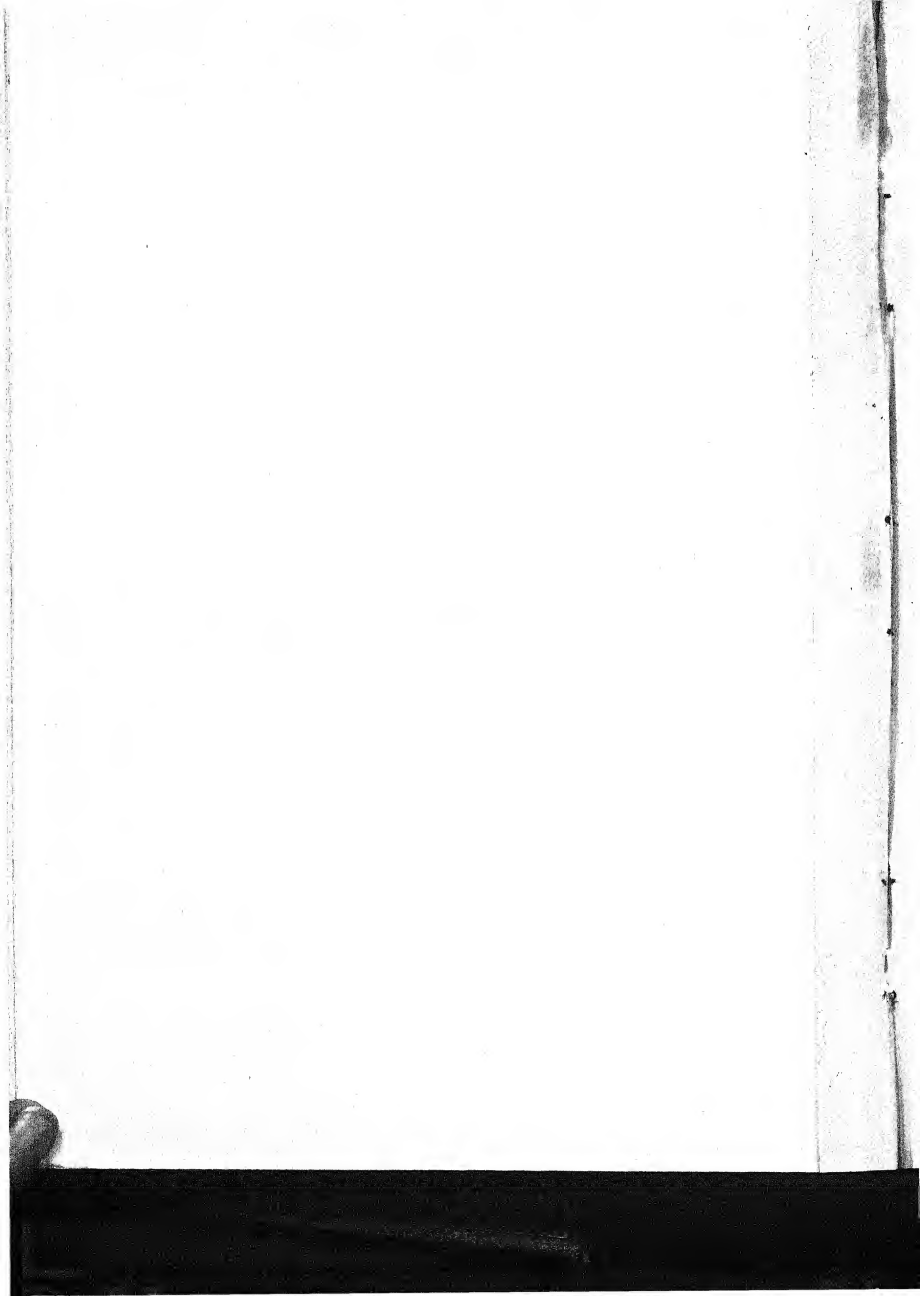
ALBUS.



SH

FIVE DOTS.

PETIOLES AND STEMS.



interaction of these three factors, together with the large number of heterozygotes produced an almost perfect series in which it was extremely difficult to state, from observation only, to which class any individual plant belonged. The following method was, therefore, adopted. Seed of all the plants (145) of one of the F_2 cultures was sown plant by plant in 1913 and observations were made on the offspring. In the following year, a large number of selected cultures were continued into the F_4 and succeeding generations by growing several plants from each. It was quite easy to recognize homozygous cultures and also cultures in which there was only a difference of one factor. In cultures in which more than one factor were concerned, although it was possible to determine the nature of the culture and the factors involved, it was not possible to assign individual plants to their correct class with any degree of certainty.

By continuing this method to the F_7 generation, it was found that the colour markings of the leaves of *intermedius* are due to the factor or factors present in *Bhagalpuriensis* (which will be termed X in future) plus three factors A, B and W. These three factors are quite independent of one another and can be inherited separately. The presence of A converts a leaf with dots on the four outside veins into one with five dots, *i.e.*, a dot on each principal vein. Cultures breeding true to five dots were obtained and also cultures containing both four dots and five dots. The outward manifestations of B and W are somewhat alike and these two factors were at first confused with one another. More accurate observations, however, showed that they were really quite distinct, and comparatively easy to differentiate. B produces a red band on the petiole below the pulvinus. W also gives rise to some red colour on the petiole, but instead of a definite band with well-marked edges it produces a light red wash of somewhat indeterminate outline. Uniform cultures of both *band* and *wash* types were obtained and also cultures splitting between *Bhagalpuriensis* and the *band* type and *Bhagalpuriensis* and the *wash* type. These factors were also found in combination, *i.e.*, five dots and a band, five dots and wash, four dots and a band and four dots with wash. Finally all three, five dots, band and wash are present in *intermedius*. Uniform cultures of all these phenotypes were obtained and grown for some years. Most of the numerous heterozygotic cultures which should be produced by the interaction of these three factors were also identified and checked by continuing several plants from these cultures to the succeeding generation. The various combinations are shown in Plate V.

The results are summed up in the following Tables :—

TABLE VII.

Homozygotic combinations obtained from the cross Bhagalpuriensis × intermedius.

Genotype	Phenotype	Abbreviated designation
1. (XX)aabbcc	Four dots on the leaf veins, petiole green	*Bhagalpuriensis*
2. (XX)AAbbcc	Five dots on the leaf veins, petiole green	Five dots
3. (XX)aaBBcc	Four dots on the leaf veins, plus a red band on the petiole	Band
4. (XX)aabbWW	Four dots on the leaf veins, plus a red wash on the petiole	Wash
5. (XX)AABBww	Five dots on the leaf veins, plus a red band on the petiole	Band half-way
6. (XX)AAbbWW	Five dots on the leaf veins, plus a red wash on the petiole	Wash half-way
7. (XX)aaBBWW	Four dots on the leaf veins, a red band and a red wash on the petiole, *i.e.*, it resembles *intermedius* except for the absence of a red dot on the central vein	*Intermedius* with four dots
8. (XX)AABBWW	Five dots, a red band, plus a red wash on the petiole	*Intermedius*

The uniformity of these cultures was always tested by selecting several plants at random and growing the seed from them next to each other during the following year.

Cultures splitting between the following limits were also identified and tested :—

TABLE VIII.

Splitting cultures identified in the F_3 and succeeding generations from the cross Bhagalpuriensis × intermedius.

Limits of the culture	Genotype of the parent
1. *Bhagalpuriensis* to five dots	(XX)Aabbww
2. *Bhagalpuriensis* to wash	(XX)aabbWw
3. *Bhagalpuriensis* to band half-way	(XX)AaBbww
4. *Bhagalpuriensis* to wash half-way	(XX)AabbWw
5. *Bhagalpuriensis* to *intermedius*	(XX)AaBbWw
6. Five dots to band half-way	(XX)AABbww
7. Five dots to wash half-way	(XX)AAbbWw
8. Five dots to *intermedius*	(XX)AABbWw
9. Band to band half-way	(XX)aaBBww
10. Band to *intermedius*	(XX)aaBBWw
11. Wash to wash half-way	(XX)aabbWW
12. Wash to *intermedius*	(XX)aaBbWW
13. Band half-way to *intermedius* with four dots	(XX)AaBBWw
14. Band half-way to *intermedius*	(XX)AABBWw
15. Wash half-way to *intermedius*	(XX)AAbbWW
16. *Intermedius* with four dots to *intermedius*	(XX)AaBBWW

These cultures were tested in the same manner as the homozygotic ones, *i.e.*, by growing in the following year several plants from each culture separately. In these cases, instead of selecting the plants at random, care was taken to include plants representing the extreme phenotypes.

A few cultures, which theoretically should have been present, were not found; most of these involve the phenotype *intermedius* with four dots. This form is very difficult to identify and these cultures were probably confused with the corresponding ones in which *intermedius* occurred. The pure culture of *intermedius* with four dots and the splitting cultures—band half-way to *intermedius* with four dots and *intermedius* with four dots to *intermedius*—were, however, identified with certainty. The other cultures were doubtless present and either escaped recognition or did not happen to occur among the plants selected for further investigation.

TABLE IX.

Splitting cultures, theoretically required, which were not found.

Limits of culture	*Genotype of parent*
1. *Bhagalpuriensis* to band	(XX)aaBbww
2. *Bhagalpuriensis to intermedius* with four dots	(XX)aaBbWw
3. Five dots to *intermedius* with four dots	(XX)AABbWw
4. Band to *intermedius* with four dots	(XX)aaBBWw
5. Wash to *intermedius* with four dots	(XX)aaBbWW
6. Wash half-way to *intermedius* with four dots	(XX)AaBbWW

The stem markings in the axil of the leaf and below the petiole are due to the same factors which affect the red colouration of the petiole and leaf-veins. Each factor produces a change in the stem markings as well as on the leaves. The factor or factors which produce the four dots in *Bhagalpuriensis* are also responsible for the triangular red patch in the axil of the leaf. The factor A which controls the fifth dot also produces two narrow red streaks on the stem which start from the two basal angles of the triangle and run a short way down the stem. The factor B not only gives a band across the petiole but longer and larger red streaks on the stem. The factor W produces a certain amount of red colour below the petiole. The presence of all three factors together is shown by the well-marked triangular patch below the petiole in *intermedius*. In no case was it possible by breeding to separate the leaf-

markings from the corresponding stem-markings. We must, therefore, conclude that the difference in the red colouration of the leaf, petiole and stem of *intermedius* from that of *Bhagalpuriensis* is due to three factors only. The presence of each factor is shown by a definite addition to the markings both of the leaf (including the petiole) and of the stem.

3. The general colour of the stem.

It was pointed out in a previous section (p. 50) that in addition to the well defined patches of red on the stem, certain flushes or washes also occur over large portions of the stem. These vary in colour and distribution and may be roughly divided into (1) those like the crimson factor in *ruber* which obscure all other stem markings and which affect other portions of the plant simultaneously and (2) those which are light in colour, which allow the stem markings to be identified and affect the stem only.

(*a*) *The crimson colour factor of ruber.*

The next factor which must be considered is the one which is responsible for producing the red colour over the stem and the major portion of the plant. This crimson flush, as it may be called, has a very far reaching effect and whenever it is present, the whole plant would be classed as crimson or scarlet. In all crimson types there is a certain amount of green, the distribution and amount depending on the genetic constitution of the type. For instance, in the F_1 between *intermedius* and *albus*, the green base of the stem was more extensive than in *ruber* and the lower eighteen inches of the basal branches were also green. Variations in the amount of green in the petioles, leaves, epicalyx and peduncle occur in types which are nevertheless crimson. Whatever the constitution, however, if the stem is red, the branches and sepals are also red and the corolla changes to a deep pink on withering. A red calyx is inseparable from a red stem. The following experiments show that this crimson flush, which converts a green plant with red markings into a crimson one, is produced by a single factor.

The first cross made in this connection was that of *ruber* × *intermedius* *i.e.*, the cross between a crimson type and the green type which possesses the greatest amount of red markings yet observed. The F_1 was crimson and resembled *ruber*, except that more green was present and the crimson colour was slightly less intense. In the F_2, crimson plants and green plants, with the same red markings as those of *intermedius*, were obtained. It was possible to divide the crimson plants into two classes, those which were as deeply crimson as *ruber* and those in which the stem colour was lighter in tone.

The results obtained are given in the following Tables :—

TABLE X.

The F_2 generation of the cross ruber × intermedius.

No. of culture	Total No. of plants	Colour of stem		
		Deep crimson	Crimson	Green with red markings
M	77	21	39	17
N	35	9	14	12
O	47	10	23	14
P	76	22	38	16
TOTAL	235	62	114	59
Ratio		0·95	1·93	1
Expectation		*58·75*	*117·50*	*58·75*

TABLE XI.

The F_3 and F_4 generation of the cross ruber × intermedius.

	No. of culture	Nature of the parent	Nature of the offspring
F_3	M 3	green with red markings	all green with red markings
	M 13	Do.	Do. Do.
	M 44	Do.	Do. Do.
	M 70	Do.	Do. Do.
	M 71	Do.	Do. Do.
	M 1	crimson	10 green with red markings : 26 crimson : 12 deep crimson
	M 2	Do.	10 green with red markings : 21 crimson : 11 deep crimson
	M 10	Do.	green with red markings, crimson, deep crimson
	M 11	Do.	Do. Do.
	M 14	deep crimson	all deep crimson
	M 43	Do.	Do.
	M 51	Do.	Do.
	M 54	Do.	Do.
	M 56	Do.	Do.
F_4	M 1–7	green with red markings	all green with red markings
	M 1–4	crimson	143 deep crimson and crimson 36 green with red markings
	M 1–3	deep crimson	all deep crimson
	M 13–1	green with red markings	65 plants, all green with red markings
	M 14–1	deep crimson	65 plants, all deep crimson

Similar results were obtained in the cross *ruber* × *Bhagalpuriensis*. The F_1 was a crimson plant slightly less deep in colour than *ruber* and differing in various details of the colouring. In the F_2, both crimson plants and green plants with red markings were obtained in the following proportion—97 crimson plants to 28 green plants with red markings, a ratio of 3·4 : 1. Thus the evidence from these two crosses indicates that the crimson flush is produced by a single factor which may be termed S.

It was shown in the last section (p. 55) that *albus* possesses the factor for crimson flush, although, owing to the absence of R, it cannot find expression. The crosses with *albus* should, therefore, also be considered in this connection.

Intermedius × *albus*. The F_1 was crimson and somewhat similar to *ruber*. The F_2 gave 99 colourless plants, *i.e.*, those in which the factor for colour production is absent and 294 coloured. The coloured plants consisted of 208 plants predominantly crimson and 86 green plants with red markings giving a ratio of 2·4 : 1. The green plants bred true to green while the crimson plants and green plants with red markings either bred true or split in various ways.

Bhagalpuriensis × *albus*. Here again the F_1 was crimson and somewhat similar to *ruber*. The F_2 gave 211 colourless plants and 645 coloured. These consisted of 492 predominantly red plants and 153 green plants with red markings, the ratio being 3·2 : 1.

The above investigations point to the existence of a factor S present in *ruber*, which covers the stem, branches, sepals and other parts of the plant with a crimson flush, obscuring all other red markings. This factor is absent in all the other three varieties. If SS or Ss is present, the plant is predominantly crimson.

(*b*) *The flushes on the stems of the cross intermedius* × *Bhagalpuriensis.*

The inheritance of the light flushes which do not obscure the stem markings (*i.e.*, the brown and pink flushes on the stem of *Bhagalpuriensis* and *intermedius*) must now be discussed. These consist of a pink flush in *intermedius* which starts at the base and extends two-thirds up the stem and lower branches and a very slight brown flush on the apices of the stem and branches. In *Bhagalpuriensis* there is a purplish brown flush at the extreme base of the plants and at the apex a flush similar to that of *intermedius* but of a deeper brown and more extensive for it is found over the upper half or third of the stem. The inheritance of these flushes is very difficult to investigate on account of their reaction to changes in the environment, especially to age and sunlight. In the first place. they cannot be seen on young plants but must be

observed on mature individuals. As the plant gets old, however, there is a general reddening of the stem, leaves and petioles which is quite independent of and obscures these definite flushes. In the second place, these flushes will only develop well in a good light. They remain poorly developed in shade. Very intense sunlight, on the other hand, produces the same reddening effect as age. For these reasons, the time during which observations can be made is very short and it has not been possible, up to the present, to investigate these characters fully. There is no doubt, however, that these flushes represent definite characters which are inherited. Preliminary investigations on a large number of crosses between the four original varieties themselves and with extracted homozygotes showed that the occurrence of these flushes is governed by two sets of factors, the one controlling the colour (pink or brown), the other the distribution. Corresponding to the minimum amount of stem markings X, a small light brown apical flush is always present, although the rest of the stem may be pure green. No individual has yet been observed without at least a small apical flush. The factors affecting the colour occur either singly or in combination when new homozygotic phenotypes are produced. Thus in the F_2 of the cross *intermedius* × *Bhagalpuriensis*, not only were plants like the parents obtained but also a series which exceeded the parental limits. The plants in this series varied from individuals without any flush (except the small apical one) to some which were completely covered by a deep purplish brown flush. Plants possessing this deep purplish brown colour over the whole plant bred true to this colour and also to this distribution. Individuals were also found with this colour but only on the upper half, *i.e.*, with the same distribution as that of the apical flush of *Bhagalpuriensis* and a culture was identified which bred true to this deep colour but in which the distribution varied from a complete colouration to a colouration of the upper portion only.

The basal flush of *intermedius* and the apical flush of *Bhagalpuriensis* are complementary to one another in distribution and the presence of both of these would give a completely coloured plant. The purplish brown colour is presumably due to the combination of the pink and brown colours of these two varieties. It seems probable, therefore, that *intermedius* possesses a factor P_1 for pink and *Bhagalpuriensis* another N for brown, the combination P_1N being a purplish brown much deeper than either.

The question of the interaction of these flushes with the scarlet flush S is somewhat difficult to investigate, also the constitution of *ruber* in this respect. There seems no doubt that *ruber* does not contain either the pink flush of *intermedius* or the brown flush of *Bhagalpuriensis*. It appears,

however, to possess a factor controlling a second brown flush which is less purple in tone than that of *Bhagalpuriensis*. Evidence of the absence of the pink flush and the presence of a brown flush was obtained from the cross *ruber* × *intermedius* of which the F_2 was subjected in 1922 to a critical examination. About seventy-five per cent of the plants were predominantly crimson and the remainder consisted of plants with green stems and the red stem and leaf markings characteristic of *intermedius*. The stem flushes on these green stemmed plants varied very considerably both in colour and distribution ; pink, light-brown, dark-brown and various intermediates were present. Certain plants were completely covered by a dark-brown flush. Individuals either with the true pink of *intermedius* or with a dark brown flush were very few in number, the majority had flushes of varying tints of pink and brown, mostly apical. The deep brown flush produced in this cross is quite different in appearance from the purplish brown one obtained in the case of *intermedius* × *Bhagalpuriensis* and the light brown flushes are also much less deep in colour than those of *Bhagalpuriensis*. The variation in the colour of the flushes in the F_2 and the presence of individuals without the pink flush point to the absence of P_1 in the genotype of *ruber*. The presence of individuals with light brown and deep brown flushes indicates the presence of a brown flush M in *ruber*. That this brown flush is not the one present in *Bhagalpuriensis* is indicated by the difference in the tone of the browns. Further evidence on this point is afforded by the cross *ruber* × *Bhagalpuriensis*. Before leaving the discussion of the cross *ruber* × *intermedius* it may, however, be mentioned that the predominantly crimson plants varied in the tone and amount of crimson colour present. This is what would be expected if S were interacting with P_1, M and the factors for distribution.

A consideration of the cross *ruber* × *Bhagalpuriensis* shows that *ruber* does not possess the brown flush of *Bhagalpuriensis*. In the F_2 it was comparatively easy to separate the plants containing SS, *i.e.*, those which would breed true to crimson from those containing Ss which would throw a certain number of plants with green markings. All the cultures were continued into the F_3 when this classification was found to have been correctly made. The SS. plants, however, varied very greatly among themselves both as regards tone and distribution of colour. Some were even more deeply coloured than *ruber* while others were scarlet rather than crimson.

If *ruber* contains S and M and *Bhagalpuriensis* N, we should expect at least four different tones of colour SMn, SMN, SNm and Snm. In addition to these four tones of colour, differences of distribution would have an effect and the number of scarlet or crimson phenotypes breeding true to S would be large.

It was possible to isolate at least three homozygotic forms which were predominantly crimson and which yet were not *ruber*. The first was a form termed *purple* which was almost indistinguishable from *ruber*. The most striking difference was the continuance of the colour to the extreme base of the plant whereas in *ruber* there is a little green at the base. Very careful examination showed that the general colour was very slightly darker than that of *ruber* and this was confirmed by examination of sections under the microscope. The tone of colour by itself was not, however, sufficient to differentiate the two forms by a casual examination. These purple forms bred true or split into a mixture of *ruber* and purple. They were only found when *ruber* or *albus* was crossed with *Bhagalpuriensis*. This purple form must represent therefore the intensification produced by the combination of the flushes in *Bhagalpuriensis* with the flush or flushes of *ruber*. Two other forms were isolated in this cross (*ruber* × *Bhagalpuriensis*) and bred true. These differed from *ruber* in the other direction, the colour was a clearer scarlet and less purple while the amount of green at the base and on the plant generally was much increased. These two forms were termed O and X. In O, the lowest foot of the stem and the lowest foot of the main branches were green with splashes of red. The upper portion of the stem and branches were like *ruber*. The petiole was green at the back, red in front and at the base whereas in *ruber* it is red with a dash of green; the bracts also were greener. In the form X, there was much more green present; the lower half of the stem and branches were green with red splashes on the branches. These splashes increase in size until the top of the stem and branches are quite red. The petiole is like X but with less red.

The large number of grades in the tone of colour of the crimson plants in the F_2 of this cross, the extraction of homozygotic forms both darker and lighter in stem colour than *ruber* undoubtedly indicate that the stem flush of *Bhagalpuriensis* is not contained in *ruber* but is controlled by an independent factor. The results obtained in the cross *albus* × *Bhagalpuriensis* agree with this.

These preliminary investigations thus indicate the presence of four factors governing the tone of colour of the stem, namely, S a scarlet flush, M a brown flush present in *ruber*, N a brown flush with a purple tinge present in *Bhagalpuriensis* and P_1 a pink flush present in *intermedius*. A good deal more work is required to confirm and finally establish these results.

During the course of these experiments, evidence was also obtained that besides differences in colour, factors governing distribution are involved. U to the present, it has not been possible to define the factors more accurately

and very large cultures would probably be necessary for the solution of these problems.

4. The corolla.

In all the varieties except *albus*, the corolla is yellow with a crimson eye. Just as it was impossible to obtain any plant without the factor for a certain minimum amount of red colour on the leaves and stem, so it was impossible to produce any type without the factor for the crimson eye. Var. *albus* and other green phenotypes all possess this factor, although it cannot always find expression. There is a slight difference in the depth of eye colour between *Bhagalpuriensis* and *intermedius* but it is so small that a detailed investigation proved to be impossible. In the F_1 of the cross between these two varieties, the eye was as deep a crimson as that of *intermedius*. In the F_2, all the flowers had crimson eyes showing that both varieties possess the same factor for crimson eye and that the difference must be produced by some factor of intensification or the reverse.

There is only one other character of the corolla in which the four varieties differ from one another and that is the colour assumed by the petals while fading. The flowers of this species open late in the morning and close about midday, remaining open for not more than three hours. The closing of the flower is fairly rapid and the petals become twisted after the flowers have closed. During this period, a change takes place in the colour in some varieties, the yellow petals turning salmon pink. This occurs in *Bhagalpuriensis* and in *ruber* but the intensity of the colour is not the same in both. It is much paler in *Bhagalpuriensis*. The petals of *intermedius*, on the other hand, never turn pink. They remain yellow until they wither.

Bhagalpuriensis × *intermedius*.

In the F_1, the corolla on fading turned a very faint pink and was thus intermediate between the parents. The F_2 consisted of a mixture of plants with yellow-fading and pink-fading corollas in approximately the ratio of 1 : 3. Those with pink-fading corollas could be divided into two classes, *i.e.*, those resembling *Bhagalpuriensis* and those resembling the F_1. The plants with yellow-fading corollas invariably bred true to this character in the F_3 and in succeeding generations. Those with pink-fading corollas of the deeper hue also bred true to this character while the more faintly coloured ones gave a mixed progeny of plants with yellow and pink-fading corollas.

TABLE XII.

Inheritance in the F_2 of changes in the corolla colour in the cross Bhagalpuriensis × intermedius.

No. of culture	No. of plants	Corolla on fading pink	Corolla on fading pale pink	Corolla on fading yellow
I	83	24	43	16
II	88	28	45	15
III	144	34	75	35
IV	147	30	88	29
V	128	21	79	28
TOTAL ..	590	137	330	123
RATIO ..	....	1	2·4	·9
Expectation ..	....	*147·5*	*295·0*	*147·5*

That the division into pink and pale pink was not quite accurate is shown by the results of the F_3 generation.

TABLE XIII.

Inheritance in the F_3 of changes in the corolla in the cross Bhagalpuriensis × intermedius.

No. of culture	COLOUR OF COROLLA ON FADING		RATIO
	Parent	Offspring	Pink : yellow
1	yellow	yellow	
2	Do.	Do.	
3	Do.	Do.	
4	Do.	Do.	
5	pale pink	pink, pale pink and yellow	
6	Do.	Do.	
7	Do.	Do.	
8	Do.	Do.	
9	Do.	Do.	
10	Do.	Do.	
11	Do.	Do.	
12	Do.	Do.	401 pink to 151 yellow 2·7 : 1.
13	Do.	Do.	
14	Do.	Do.	
15	Do.	Do.	
16	Do.	Do.	
17	Do.	pink	
18	pink	Do.	
19	Do.	Do.	
20	Do.	Do.	
21	Do.	Do.	
22	Do.	Do.	
23	Do.	Do.	
24	Do.	pink, pale pink and yellow	

The factor which produces this pink colour in the corolla is also responsible for the red markings in the full grown calyx. In this species, the fruit is covered during the whole period of its development by the fleshy sepals which increase in size with the expansion of the capsule. In both the varieties under discussion, the sepals are green at the apices and at the base and yellowish green to colourless in the middle. In *intermedius*, there is no red colour on the calyx, while in *Bhagalpuriensis* the sepals are splashed with bright red (Plate VI). In the F_2 of the cross between these two varieties, all the plants in which the corolla remained yellow had calyces without any red colouration. This was found to hold in all succeeding generations. The remaining plants of the F_2 all possessed calyces splashed with red but the amount of red colour varied from a few dots to something much more intense than the markings on the capsules of *Bhagalpuriensis*. In all these plants, whatever the amount of red on the calyx, the corolla turned pink on fading. The change in corolla colour is, therefore, associated with the existence of red colour in the calyx. It is not connected either with the amount or with the distribution which are controlled by other factors.

Ruber × *Bhagalpuriensis*.

As stated above, the corolla of both these varieties turns pink but the colour is much deeper in the case of *ruber*. In the F_1 between these two varieties, the faded corolla was pink also. The tone of colour was intermediate between that of the two parents. In the F_2, all the corollas turned pink on fading and the same was the case in the F_3 and succeeding generations. As would be expected from the observations in the cross *intermedius* × *Bhagalpuriensis*, all the calyces in the F_2 were either red or splashed with red. Thus the factor F which is present in *Bhagalpuriensis* and which is responsible for the change in the corolla colour and the production of red markings on the calyces, must also be present in *ruber*. This latter variety, however, possesses some additional factor which produces the intensification of the colour in the fading corolla. The factor F is either absent or suppressed in *intermedius*.

Similar results were obtained in the cross *albus* × *Bhagalpuriensis*. All the green plants with red markings possessed pink fading corollas and calyces splashed with red.

5. The colour of the calyx.

(*a*) *The sepal tips.*[1]

The first character which must be dealt with is the general ground colour of the sepal which is best seen in the mature tips. In *albus*, the tips are yellow,

RUBER

INTER.

BHAG.

ALBUS.

BRIGHT GREEN.

GREEN.

OLIVE GREEN.

STRONG FLUSH.

RED DOTS

PINK.

RED

THE COLOURS OF THE MATURE CALYX.

in *intermedius*, green, in *Bhagalpuriensis*, bright green. These differences are very distinct and are well shown in Plate VI. The relation between green tips and bright green tips was studied in the cross *intermedius* × *Bhagalpuriensis*. The F_1 was intermediate between the two parents. Beyond ascertaining that the tips of the sepals were of three tones of green, the F_2 was not further investigated. In the F_3 and F_4, however, detailed observations were made in most of the cultures. The results are given in the following Table :—

TABLE XIV.

Inheritance in the F_3 and F_4 of bright green and green sepal tips in the cross intermedius × Bhagalpuriensis.

A. Cultures which split into green and bright green.

No. of culture	Total No. of plants	Colour of sepal tips		Nature of the red markings on the sepals
		Bright green	Green like *intermedius* and the F_1	
III-3-8	66	16	50	varied from none to markings like those of *Bhagalpuriensis*
III-3-18	66	18	48	like *Bhagalpuriensis*
III-4-3	54	9	45	like *intermedius*
III-4-5	7	1	6	Do.
III-4-14	4	1	3	Do.
III-4-24	18	7	11	Do.
III-6-29	60	11	49	varied from none to markings like those of *Bhagalpuriensis*
III-7-2	34	6	28	varied from markings like *Bhagalpuriensis* to something more intense
III-13-4	27	7	20	varied from markings like those of *Bhagalpuriensis* to something more intense
III-13-7	28	6	22	like *Bhagalpuriensis*
III-13-21	27	7	20	all more intense than those of *Bhagalpuriensis*
III-13-25a	20	5	15	Do.
III-13-43	26	6	20	all more intense than those of *Bhagalpuriensis*
III-13-1	25	6	19	varied from markings of *Bhagalpuriensis* to something more intense
TOTAL	462	106	356	
RATIO		1	3·3	
Expectation		*115·5*	*346·5*	

B. Cultures which bred true in the F_3 and F_4 to green and bright green.

CULTURE	F_3 GENERATION			F_4 GENERATION	
	Total No. of plants	Red markings on the sepals	Colour of the sepal tips	No. of cultures grown	Colour of the sepal tips
III-4-2	39	almost colourless	all green	4	all green
III-4-48	14	like those of *intermedius*	Do.	2	both green
III-5-28	17	all more intense than those of *Bhagalpuriensis*	Do.	2	Do.
III-5-32	?	Do.	all green	3	all green
III-5-30	7	Do.	Do.	–	—
III-6-30	34	Do.	Do.	4	all green
III-7-4	33	like those of *Bhagalpuriensis*	Do.	3	Do.
III-10-36	32	like those of *intermedius*	Do.	–	—
III-10-34	28	Do.	Do.	–	—
III-10-42	29	Do.	Do.	2	all green
III-10-44	28	Do.	Do.	–	—
III-10-78	31	Do.	Do.	–	—
III-13-3	35	varied from none to markings like those of *Bhagalpuriensis*	Do.	–	—
III-3-22	66	Do.	all bright green	–	—
III-6-30	34	more intense than those of *Bhagalpuriensis*	Do.	2	all bright green
III-6-31	33	like those of *intermedius*	Do.	–	
III-7-3	25	Do.	Do.	4	all bright green
III-9-1	34	colourless	Do.	–	Do.
III-9-12	25	Do.	Do.	3	Do.
III-9-13	29	Do.	Do.	5	Do.
III-9-39	22	Do.	Do.	2	Do.
III-9-83	37	like those of *intermedius*	Do.	2	Do.

It will be seen from this Table that bright green behaves as a simple allelomorph to green. It is also clear that the shade of green in the tip is quite independent of the red markings on the sepal. It is possible to get the red markings of *Bhagalpuriensis* combined with the green tips of *intermedius* and vice versa. Two interpretations are possible. We may assume that the green of the sepal tips in *intermedius* is the normal and that the bright green tips are produced by the addition of a factor G. On the other hand, the bright green tips may be normal and the more yellowish green tips of *intermedius* may be produced by a colour restraining factor I which is only present in the flower and capsule and does not affect the rest of the plant. Taking into consideration the fact that the bright green of the sepal tips in *Bhagalpuriensis* is the same shade as that of the epicalyx and of the plant in general, the latter seems the more probable explanation.

The cross *intermedius* × *albus* is an example of green tips × yellow tips but the investigation is somewhat complicated by the red factor which is latent in *albus*. As stated on page 53, the F_1 is crimson and the F_2 a mixture of crimson plants, green plants with red markings and green plants with no red colour. Observations on the crimson plants as regards this character are, of course, impossible. All the green plants with red markings had sepals with green tips like *intermedius*, while the sepal tips in the plants with no red colour were either green, yellow or a shade of green intermediate between these. The sepals with yellow tips formed about one-quarter of the whole number—ratio 1 : 2·7. In the F_3, cultures breeding true to green tips and to yellow tips were found; also cultures which again split into all three forms. The yellow sepal tips are only to be found among plants with no red colour (*i.e.*, those in which R is absent); the green plants with red markings all had green sepal tips. Further observations in subsequent generations confirmed this point. The factor which produces yellow tips is therefore dependent on the absence of R.

Further information regarding the constitution of the yellow sepal tips was obtained from the cross *Bhagalpuriensis* × *albus*. Here again yellow tips were only found on plants which were entirely green. In the plants with red markings, the sepal tips were either green, bright green or a shade intermediate to these. In the F_3 and F_4, four homozygous phenotypes were obtained from the entirely green plants, *i.e.*, bright green tips, green tips, yellow tips and a new form with olive green tips. Cultures splitting between these limits were also found. The detailed results as regards sepal tips of all the entirely green plants (22) produced in the F_2 of this cross are given in Table XV.

TABLE XV.

Inheritance of sepal tip colour in the F_3 and F_4 of the cross Bhagalpuriensis × albus.

F_3 GENERATION 1913–14			F_4 GENERATION 1914–15	
			Colour of the sepal tips	
No. of culture	Colour of the sepal tips	No. of culture	Parent	Offspring
A5	14 bright green 26 intermediate 12 olive green	A5–1	bright green	all bright green
		A5–2	olive green	all olive green
		A5–20	intermediate	split bright green to olive green; out of 18 plants 5 were bright green
A10	yellow tips to olive green tips 5 olive green plants in 17	A10–3	olive green	all olive green
		A10–4	intermediate	split into yellow, olive green and intermediate
		A10–5	olive green	all olive green
A11	7 yellow; 14 intermediate; 7 olive green	A11–2	intermediate	3 olive green 10 (F_2 + yellow)
		A11–5	olive green	all olive green
		A11–19	yellow	all yellow
A19	all olive green	A19–1	olive green	all olive green
		A19–2	Do.	Do.
A28	all green	A28–1	green	all green
		A28–2	Do.	Do.
A35	all bright green	A35–1	bright green	all bright green
		A35–2	Do.	Do.
A39	splitting culture containing among other types both bright green and yellow	A39–8	bright green	all bright green except 1 red plant probably a cross
		A39–21	yellow	all yellow

TABLE XV.—*contd.*

F_3 GENERATION 1913-14		F_4 GENERATION 1914-15		
			Colour of the sepals tips	
No. of culture	Colour of the sepal tips	No. of culture	Parent	Offspring
A45	all olive green	A45–1	olive green	all olive green
		A45–2	Do.	Do.
A48	all yellow ..	A48–1	yellow	all yellow
A51	Splitting culture containing yellow, bright green, olive green and green	A51–3	intermediate	splitting culture, 4 olive green : 9 intermediate : 5 yellow
		A51–4	yellow	all yellow
		A51–7	bright green	all bright green
A83	all green	A83–1	green	all green
A58	all olive green	A58–1	olive green	all olive green
A79	all yellow	A79–1	yellow	all yellow
A13	splitting culture	....	....	....
A22	splitting culture	....	....	....
A24	splitting culture	....	....	....
A31	splitting culture	....	....	....
A49	splitting culture	....	....	...
A54	splitting culture	....	....	....
A72	splitting culture	....	....	....
A78	splitting culture	....	....	....
A82	splitting culture	....	....	....

Out of 22 cultures, the following bred true, 1 bright green, 3 olive green, 2 green and 2 yellow. Cultures splitting from yellow to olive green and giving a ratio of approximately 3 : 1 were obtained and a similar culture splitting from bright green to olive green. Cultures containing bright green, yellow, olive green, green and various intermediates were also identified. These results show that in the production of yellow tips another factor

must be involved in addition to I, the factor which reduces bright green tips to green tips. The olive green tips are due to the action of this factor (which may be termed Y) on bright green tips. If we denote the factor or group of factors which is responsible for the bright green of the sepal tips G, the following combinations would be obtained :—

Bright green	GGiiyy
Green	GGIIyy
Yellow	GGIIYY
Olive green	GGiiYY

Yellow or olive green sepal tips are, however, only found in entirely green plants, *i.e.*, in the absence of R. Yellow tips are also characteristic of *albus* which was shown to be a crimson plant in which the colour was not developed, *i.e.*, in which S. is present and R absent. It is possible that YY is, therefore, nothing more than the combination SSrr. This would explain the 3 : 1 ratio. The green plants occurring in the F_2 of crosses with *albus* must contain this factor SS in the following proportions 1 SS : 2 Ss : 1 ss. The various sepal tips would, therefore, be represented by the following combinations :—

Bright green	GGiissrr
Green	GGIIssrr
Yellow	GGIISSrr
Olive green	GGiiSSrr

The combination GGiissRR would also be bright green and GGIIssRR would be green while GGIISSRR and GGiiSSRR would be crimson.

(*b*) *The red colour on the sepals.*

In addition to the changes in the tone of green, the capsules of the four varieties differ very markedly in the amount of red colour on the sepals. The capsules of *ruber* are crimson all over, those of *Bhagalpuriensis* have red splashes on the upper portion, while those of *albus* and *intermedius* appear, at first sight, both to be entirely green. A closer examination shows that this is only true of *albus*. The sepals of *intermedius* have a flush of very faint pink dots on the lower portion of the sepal (Plate VI). As would be expected, no flush is found in plants with absolutely green stems, *i.e.*, those in which R is absent. Plants with red markings on the stem always have some red on the sepals even though it may only be a slight flush as in *intermedius*. As shown before in section 4 (p. 66) distinct red markings on the sepals are always associated with a salmon pink colour of the faded corolla. When the sepals show only the flush, the corolla remains yellow on withering. Neither the red

markings nor the flush, however, show any connection with the amount of red on the stem nor with the tone of green of the sepals (p. 69). It has not been possible to work out in detail the factors governing the nature of the red markings on the sepals but they seem to be governed by factors regulating both the colour and the distribution as was the case with the flushes on the stem. In the F_2 of *intermedius* (green except for faint pink dots on the lower part of the calyx) $\times$ *Bhagalpuriensis* (red markings on the upper portion) a very large number of forms are obtained, many of them much more heavily marked than *Bhagalpuriensis*. On certain plants, the sepals are so heavily marked with red that the whole fruit is almost covered (Plate VI). These forms breed true in succeeding generations. They show two different tones of colour: (1) pink like the flush on *intermedius* and (2) red like the markings of *Bhagalpuriensis*. No calyces are found without any colour but some appear to have a fainter flush even than *intermedius*. *Intermedius* would thus appear to possess a factor for pink which is prevented from expression owing to the absence of F, the factor which produces the colour of the sepals and fading corolla. *Bhagalpuriensis* contains another factor which produces the red tone. *Intermedius* also possesses a pink stem flush but the connection (if any) between the pink colour on the capsule and the pink colour on the stem has not yet been worked out.

In addition to these two factors for colour, there must be factors controlling the distribution. The colour on *Bhagalpuriensis* is confined entirely to the upper half of the sepals, the faint pink dots on *intermedius* occur only on the lower portion, but the more highly coloured fruits extracted from the F_2 are almost completely coloured. The application of the colour also seems to vary. It may occur as distinct dots or as larger splashes. This difference is well shown in Plate VI. There was a certain amount of variation in the distribution of the dots. In some, these were only found on the upper portion of the capsule, as in *Bhagalpuriensis*, but there were no splashes of continuous colour such as occur in that variety. In addition, individuals were found with a stronger pink flush than in *intermedius* (Plate VI). As the disentangling of these factors necessitated more time and land than we have had at our disposal recently, or are likely to have in the near future, it seems best to record these observations now.

III. SUMMARY.

For the sake of clearness, the results obtained by crossing the four varieties may be given in a general form. This will enable some idea to be formed of the genetic constitution of these varieties.

1. *Intermedius* × *Bhagalpuriensis.*

BHAGALPURIENSIS.

Stem. Green with a red triangle above the petiole, brown flush over the upper half of the plant and over the base.

Leaf. Four dots on the back of the outer veins on the underside.

Corolla. Pink-fading.

Sepals. Tips bright green, red markings (splashes and dots) on the upper portion of the mature sepal.

INTERMEDIUS.

Stem. Green with two red triangles, one above and one below the petiole, a pink flush on the lower two thirds of the stem, a very slight brown flush at the apex.

Leaf. Five dots on the veins on the under side; a band of red below the pulvinus, the pulvinus red.

Corolla. Yellow-fading.

Sepals. Tips green, very faint pink dots on the lower portion of the mature sepals.

F_1.

Intermediate in all respects between the two parents.

F_2.

Stem and leaf. All the plants were green with red markings. Nothing was produced outside the limits of the parents. Six intermediate homozygotic forms were found due to the addition of the factors A (fifth dot), B (red band below the pulvinus) and W (red wash over the pulvinus) to X the factor or group of factors present in *Bhagalpuriensis.* These three factors which are quite independent of all other colour factors, each control a certain amount of red colour below the petiole and together form the red triangle present in *intermedius.* As regards the coloured flushes, a slight brown apical flush was observed in all plants derived from this cross. If this apical flush be left out of account, several plants had clean green stems, others were completely covered by a dark purplish brown flush which was shown to breed true. This represents the union of the pink flush of *intermedius* with the brown flush of *Bhagalpuriensis.* These results would point to the existence in *intermedius* of one factor for the pink colour P_1 and another for the distribution and in *Bhagalpuriensis* one factor for the colour N and one or two factors for the distribution.

Corolla. A mixture of pink, pale pink and yellow-fading corollas were obtained in the proportion of 1 : 2 : 1 approximately. Of these, the first and the third bred true, the rest split as before. This change in colour of the corolla on fading was always associated with the presence of colour on the mature calyx but was independent of the amount and also of the stem markings and stem flushes. The presence of red colour in the mature sepals and consequently the pink of the faded corolla are controlled by a single factor F.

Sepals. The colour of the tips varied from green to bright green, the numbers indicating a difference of one factor. Thus if bright green be G, green can be represented by GI.

As regards the colour on the mature capsules, these varied from faint pink dots below like those of *intermedius* or less to a colouration of almost the whole calyx the tone of colour being both pink and red. It is suggested that *intermedius* possesses a factor P for pink plus a factor T for the expression of the colour on the lower half of the calyx while *Bhagalpuriensis* possesses a factor D for red and a factor Q for distribution on the upper half of the calyx. Other factors regulating the appearance of the colour as dots (L) or as splashes (K) are probably also present.

A tentative genetic constitution for *Bhagalpuriensis* would therefore be :—RsFXabwp$_1$mNGipDtQLK plus factors for distribution of the flushes on the stem. For *intermedius* the following is indicated :—RsfXABWP$_1$ mnGJPdTqLk plus factors for distribution of the stem flushes.

2. *Ruber* × *intermedius.*

Ruber.

Plants with a crimson flush over stem, petiole, leaf-veins, peduncle, epicalyx and calyx. A little green at the base of the stem. Corolla fades a deeper pink than in *Bhagalpuriensis.*

F_1.

This is also predominantly crimson (slightly less intense than in *ruber*) but the base and the lower eighteen inches of the branches are green with red splashes; the leaf-veins, peduncle and epicalyx have splashes of green; the calyx is crimson but it is slightly less intense in colour than in *ruber.*

F_2.

Crimson plants and green plants with red markings on the stem and leaves were obtained in the proportion of 3 : 1. As regards the red markings

on the stem and leaves, the colour of the sepal tips, the faint pink flush on the sepals and the colour of the corolla on fading, the extracted green plants were exactly similar to *intermedius*. Differences were, however, observed as regards the stem flushes. These varied from pink to brown but nearly all the plants had a decided flush at the apex. *Ruber* possesses a brown flush M (probably apical) which is not present in *intermedius*. As regards the crimson plants, these varied in the distribution of the colour. About one-third bred true to red, the others split giving crimson plants and green plants with red markings in the proportion of 3 : 1.

Ruber would thus appear to have the same constitution as *intermedius* except as regards the factors governing the stem flushes. *Ruber* lacks the factor for the pink stem flush of *intermedius* but possesses the factor S which produces a crimson colour over the stem and the whole of the calyx and also possesses a factor M for a brown stem flush.

3. *Ruber* × *albus*.

Albus.

The plants are entirely green but of a yellowish tone. The sepal tips are yellow. There is no red colour on any part of the plant, even the eye of the corolla is colourless and the pollen is yellow.

The F_1 between these two varieties is indistinguishable from *ruber*. In the F_2, plants resembling *ruber* and *albus* were produced in the proportion of 3 : 1. In the F_3 and succeeding generations, the green plants gave only green offspring, some of the crimson plants bred true, the others split into crimson : green : : 3 : 1. Nothing different to *ruber* and *albus* was produced in this cross. All the green plants had yellow sepal tips.

4. *Albus* × *intermedius*.

F_1.

This resembles the F_1 between *ruber* and *intermedius*.

F_2.

Three classes of plants were produced, the first consisted of crimson plants, the second of green plants with red markings and the third of plants which were entirely green. These formed about one quarter of the whole. The green plants with red markings formed one quarter of the remainder.

The green plants bred true to green in succeeding generations. They were all alike except as regards the sepal tips which split from green to yellow, the

numerical proportion indicating the difference of one factor. This factor YY is probably nothing more than the combination SSrr. The mature sepals were all devoid of the faint pink dots of *intermedius*.

The green plants with red markings resembled *intermedius* exactly except for the stem flushes. The sepal tips were all green and the mature sepals all had the faint pink dots of *intermedius*. In the next generation, about one-third of these plants bred true to everything except stem flushes; the rest split into plants with red markings and entirely green plants in the proportion of 3 : 1. The entirely green plants produced by the splitting of some of the green individuals with red markings all had green sepal tips. This is in agreement with the hypothesis that the yellow tips are produced when S is present and R absent. Unfortunately, the nature of the stem flushes on the green plants with red markings was not noted. Among the crimson plants some bred true to crimson; some split again into three classes; some gave only crimson plants and green plants with red markings (3 : 1), and some gave crimson plants and entirely green ones in the proportion of 3 : 1. As regards the coloured plants, this cross behaved exactly like *ruber* × *intermedius*.

From the results of these two crosses we may conclude that *albus* has the same constitution as *ruber* except for the absence of a factor RR which is necessary for the production of any red colour. Thus

albus plus RR = ruber.

5. *Ruber* × *Bhagalpuriensis*.

F_1.

This was predominantly crimson but with a greater amount of green than in the F_1 of *ruber* × *intermedius*.

F_2.

Crimson plants and green plants with red markings were obtained in the proportion of 3 : 1.

Green plants with red markings.

Stems and leaves. As regards these characters, the plants were not uniform as in *ruber* × *intermedius* but showed all gradations between the markings of *Bhagalpuriensis* and those of *intermedius*. *Ruber* must therefore contain the factors A, B and W which convert the markings of

Bhagalpuriensis into those of *intermedius*. This confirms the results obtained in the cross *ruber* × *intermedius*. The flushes on the stem varied from no flush to a dark brown flush which bred true. This probably represents the union of the brown flush present in *ruber* with the brown flushes of *Bhagalpuriensis*. The tone of this dark brown flush is quite distinct from that of the purplish brown flush produced by the union of the pink flush of *intermedius* with the brown flush of *Bhagalpuriensis*. The presence of this dark brown flush and the occurrence of some plants with no flush show that the brown flush of *ruber* is governed by a different factor to that controlling the brown flush of *Bhagalpuriensis*.

Corolla. In all plants, the corolla turned pink on withering showing that both parents possess the factor F for colour production in the calyx.

Sepals. The tips varied from bright green to green indicating the presence in *ruber* of the factor I. The red markings on the mature sepals varied from something less than the markings of *Bhagalpuriensis* to an almost complete colouration of the calyx and both the pink and dark red colours were present. Some of the least highly coloured calyces showed the red colour in dots only. This would indicate the absence in *ruber* of the factor which converts dots into splashes, and the presence of the factor for pink colour.

The fact that in the F_2 all the mature calyces have red markings point to F being present in both *ruber* and *Bhagalpuriensis*. On the other hand, in the cross *ruber* × *intermedius* only calyces without red markings (except for the faint pink dots) were produced. The scarlet stem flush SS is, however, unlike the other stem flushes, never found without scarlet capsules and the simplest explanation would seem to be a complete linkage of S + F whenever both are present together.

Crimson plants.

The 97 plants comprised in this class differed among themselves very widely as regards the distribution of the crimson colour and the tone of the colour. All (except 3 which were too feeble to give seed) were grown in the F_3. Thirty-four plants gave offspring which were all predominantly crimson, sixty split into crimson plants and green plants with red markings. These thirty-four plants varied among themselves as to the amount of crimson colour present. A certain number were more completely coloured even than *ruber* (especially towards the base) and the colour was a somewhat deeper purple. Some of these cultures bred true in succeeding generations, others split between this purple form and *ruber*. When this extracted purple form was crossed on to *ruber*, only red plants like the two parents were produced.

When crossed on to *Bhagalpuriensis* and *intermedius*, the purple form behaved exactly like *ruber* as regards the factor S. The purple form represents the union of *ruber* with the brown flush of *Bhagalpuriensis*. Thus if the stem flushes of ruber be represented by SM, the purple form would be SMN, where N is the brown flush of *Bhagalpuriensis*.

Another crimson form was identified and found to breed true in succeeding generations. This was slightly less crimson and more scarlet in tone than *ruber* and the lower foot of the stem was green. Indications were obtained of other homozygotic crimson forms depending on the distribution factors connected with these flushes and a pure scarlet form has been identified but has not yet been proved to be homozygous.

6. *Albus* × *Bhagalpuriensis*.

F_1.

The F_1 was crimson but with more green than *ruber* and resembled the F_1 of *ruber* × *Bhagalpuriensis*.

F_2.

Three classes of plants were obtained, crimson plants, green plants with red markings and entirely green plants. The green plants formed about one quarter of the whole, the green plants with red markings one quarter of the remainder. All the F_2 plants were continued into the F_3.

Plants entirely green.

These proved to be homozygous to this character. They differed only in the colour of the sepal tips which were bright green (GGiissRR), green (GGIIssRR), olive green (GGiiSSrr), yellow (GGIISSrr) or intermediate between these four homozygotic forms. Several plants with bright green and green sepal tips were crossed on to *intermedius* and *Bhagalpuriensis*. The F_2 gave entirely green plants and green plants with various amounts of red markings showing that the extracted green plants consisted of green plants with red markings in which RR was absent. No scarlet plants were obtained thus confirming the conclusion that yellow sepal tips denote the presence of S, the factor for scarlet. These extracted green plants when crossed among themselves or with *albus* gave, as would be expected, only green plants in the F_2.

Green plants with red markings.

Stem and leaf markings. These varied from those of *Bhagalpuriensis* to those of *intermedius*. Intermediates were found but nothing which could not

be represented by the factors X, A, B and W. Certain individuals in the F_3 produced only green plants with red markings, the others split giving some entirely green plants (3 : 1).

Corolla. In all the plants, the corolla turned pink on fading.

Sepals. The tips varied from bright green to green, indicating the presence in *albus* of the factor I. The mature sepals all had red markings. These markings varied from something less than those of *Bhagalpuriensis* to calyces which were almost completely coloured. The observations on the calyces were made at an early stage of the investigation and fuller details are unfortunately not available. As far as the notes go, the calyces seem to have resembled in their markings those obtained in the last cross.

Crimson plants.

Some bred true to crimson, others split into crimson and green (3 : 1), some into crimson and green with red markings (3 : 1) while others reproduced the F_2 generation. These crimson plants varied very considerably but could not be further analysed. The presence of the purple form produced by the union of *ruber* plus the brown flush of *Bhagalpuriensis* was, however, demonstrated.

IV. CONCLUSIONS.

The investigation of the factors governing the inheritance of the colours in *Hibiscus Sabdariffa*, although incomplete, appears to justify the following conclusions :—

1. There exists a factor R which is necessary for the expression of red or crimson colour in any part of the plant. Colour is also produced in the presence of Rr. This factor is absent in *albus*.

2. The presence of R permits the production of red colour in the stem, leaves, corolla eye and pollen. The production of red colour on the mature calyx and in the fading corolla is, however, dependent on another factor F. This factor only affects the calyx and corolla. It is absent in *intermedius* but present in *ruber* and *Bhagalpuriensis*.

3. The red markings on the underside of the leaf and on the stem (both in the axil of the leaves and below the petiole) are controlled by the same factors.

4. The factor or group of factors X responsible for the red markings in *Bhagalpuriensis* (four dots on the outer veins of the leaf and a red triangle above the petiole) occur in all the four varieties. It has not been possible to produce by hybridization a phenotype without these.

5. Three independent factors A (dot on the fifth vein), B (band below the petiole) and W (red wash on the petiole) are combined with X in the markings of *intermedius*. In addition to their effect on the leaf and petiole, each of these factors is responsible for some red on the stem below the petiole. All three together produce the lower red triangle on the stem.

6. In addition to the red markings, continuous flushes of colour are present on the stem. These flushes vary in colour and in distribution. The most conspicuous is the scarlet flush found in *ruber* which is governed by the factor S. This scarlet flush is always associated with scarlet calyces and pink fading corollas, *i.e.*, S is always linked with F. The distribution of S varies but the crimson colour is never found at the base only. The crimson colour of *ruber* is produced by the presence of a brown flush M (which can exist independently) as well as S. In addition to these two flushes, two others exist, a pink flush in *intermedius* controlled by the factor P_1 and a brown flush N in *Bhagalpuriensis*. The union of P_1 and N produces a purplish brown flush, the union of M and N a dark brown flush, the union of *ruber* (*i.e.*, SM) and N produces a purple form slightly darker than *ruber*. Although both M and N are brown they are not alike. In addition to the factors controlling the colour, these flushes are influenced by distribution factors which may in some cases be linked with the colour but this point has not been sufficiently investigated.

7. The differences in the tone of colour of the sepal tips has been investigated. Representing the bright green of the sepal tips of *Bhagalpuriensis* by G, the green of *intermedius* is due to the addition of a factor I. The yellow colour typical of *albus* has been shown to occur only when S is present and R is absent and is represented by the genotype GGIISSrr. Another homozygous tone of colour, olive green was produced during the course of the investigations. This represents the genotype GGiiSSrr.

8. The factors governing the colour on the mature calyces has not been fully investigated. There appear to be two factors for colour P representing the pink present in *intermedius* (although this is only faintly visible owing to the absence of F) and a factor D present in *Bhagalpuriensis* which converts this pink into dark red. Distribution factors, independent of the colour factors, also occur, *intermedius* probably possessing a factor T for the presence of the colour on the lower half of the capsule, *Bhagalpuriensis* a factor Q for the occurrence of colour on the upper portion only. It is possible that in the future these factors may be proved not to be simple but a group of several factors. In addition, factors are present for the mode of occurrence of the colours, the factor L present in *intermedius* producing

dots. *Bhagalpuriensis* probably possesses a factor K which converts these dots into continuous splashes or produces splashes in addition to dots.

9. The following is the list of factors suggested by these investigations :—

R a factor which is necessary for the expression of all red colour.

F a factor which allows the production of red markings on the calyx and of pink in the fading corolla.

X a factor or group of factors responsible for the red markings of the leaf and the stem in *Bhagalpuriensis*.

A a factor producing a red dot on the central vein of the leaf.

B a factor producing a red band on the petiole below the pulvinus.

W a factor producing a red wash on the pulvinus.

P_1 a factor for the pink stem flush in *intermedius*.

M a factor for the brown stem flush present in *ruber*.

N a factor for the brown stem flush present in *Bhagalpuriensis*.

S a factor for the scarlet stem flush present in *ruber*.

G a factor responsible for the bright green of the sepal tips in *Bhagalpuriensis*.

I a factor which converts bright green sepal tips into the green tips of *intermedius*.

P a factor producing pink markings on the mature calyx.

D a factor responsible for the dark red colour on the calyx of *Bhagalpuriensis*.

T a factor controlling the distribution of red colour on the lower part of the mature calyx.

Q a factor controlling the distribution of the red colour on the upper part of the mature calyx.

L a factor present in *intermedius* by which the red colour on the mature calyx appears in the form of dots.

K a factor which is present in *Bhagalpuriensis* by which the red colour on the mature calyx appears in the form of splashes.

10. From the results of hybridizing the four varieties of *Hibiscus Sabdariffa* among themselves they would appear to possess the following genetic constitutions :—

Bhagalpuriensis	RsFXabwp$_1$mNGipDtQLK.
Intermedius	RsFXABWP$_1$mnGIPdTqLk.
Ruber	R(S+F)XABWp$_1$MnGIPdTqLk.
Albus	r(S+F)XABWp$_1$MnGIPdTqLk.

All probably also contain factors regulating the distribution of the stem flushes.

It is hoped that the publication of these results, although incomplete, may be of use to future workers on this species.

PUSA:
9th November, 1923.

THE "MAHALI" DISEASE OF COCONUTS IN MALABAR.

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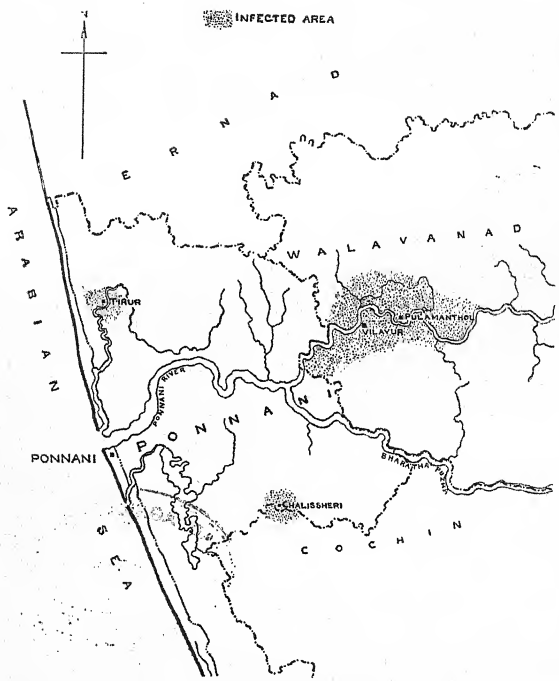
THE coconut palm is very extensively grown on the West Coast and the prosperity of the major portion of the population depends upon a good return from this crop, so much so that in some places the wealth of a man is calculated by the number of coconut trees he owns. Hence any disease which is likely to affect this palm seriously will be a heavy blow to the material welfare of the locality. In many parts of Malabar, Kanara and Mysore, the arecanut palm has been for some years past suffering from a disease called "Mahali" or "Koleroga" which causes the nuts to rot and fall off from the bunches and in extreme cases affects the crown of the palm itself. A disease resembling it in several respects has recently been noticed affecting the coconut trees in certain parts of Malabar as shown in the map. As the symptoms of the disease in both cases are similar, the garden owners in the tract call this also the "Mahali" disease.

This disease was first noticed in August 1922 after the heavy south-west monsoon in a garden at Perambalai Amsam (Cochin State) bordering on Challeseri (Ponnani Taluk). In this garden coconut palms were grown interspersed with arecanut palms. A large number of young and nearly mature coconuts were found dropped from the trees. A violent shake of the crown brought down several nuts that had rotted on the bunches. No further reports were received during the year. But after the unusually heavy rains in August 1923, reports of nut-fall were received from various places in the Ponnani and Walluvanad taluks. Generally, in these taluks various kinds

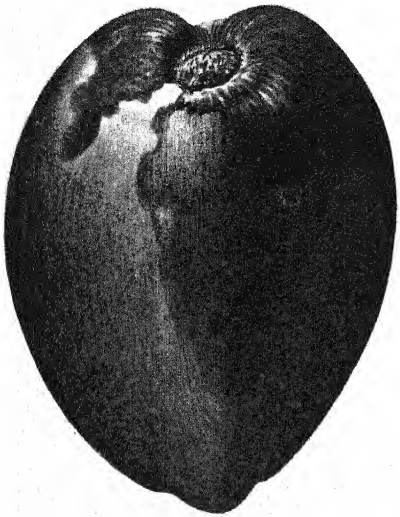
of trees are grown in one and the same garden. The coconut, arecanut and sago palms, jak and mango are all planted indiscriminately and close to one another. This year the "Mahali" disease of arecanuts was very virulent in this locality and almost every garden was affected. In the majority of the gardens the coconut palms also suffered from this.



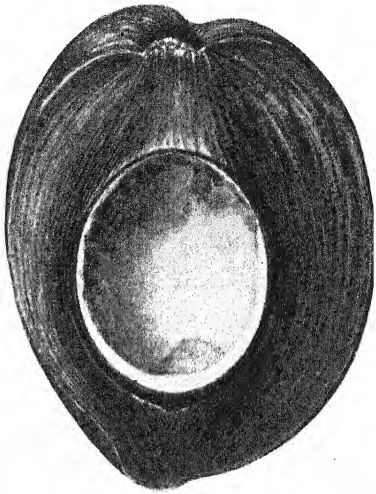
Map showing the area infected by the nut-fall of coconuts in 1923 (shaded portion).

A disease similar to this nut-fall of coconuts has been recorded by Mr. Petch[1] in Ceylon in the year 1917. It is said that an extensive fall of

[1] Petch, T. Nut-fall and leaf droop of coconuts. *Ceylon Dept. Agri. Leaflet* 6.



A



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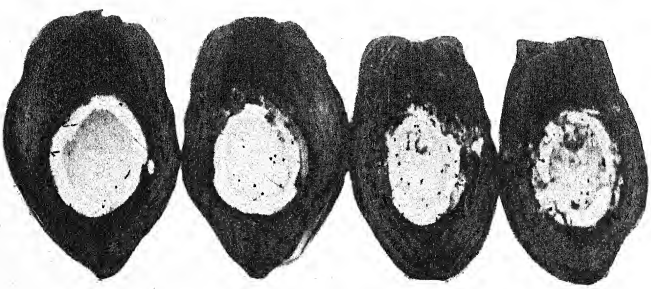

C

COCONUTS AFFECTED BY "MAHALI" DISEASE.

Explanation of Plate I.

Coconuts affected by "Mahali" disease:

A Showing discoloration on the surface with mass of whitish mycelium;

B A coconut cut open showing the contents rotten;

C Photograph of rotten coconuts cut open.

nearly mature nuts occurred after the heavy rains. The fallen nuts are found to be diseased and discoloured and a *Phytophthora* species is said to cause the disease. In 1920 Mr. Ashby[1] noticed a nut-fall in Jamaica which he attributes to a strain of *Phytophthora palmivora* Butl. Mr. Gadd,[2] Assistant Mycologist, Ceylon, comes to the conclusion that " the fall of very young immature nuts—buttons—shortly after the opening of the inflorescence must be regarded as a natural consequence of the production of an excess of female flowers. It is not normally a diseased condition though external factors such as drought may adversely affect the fall of such immature fruits. The fall of older but still immature nuts may be due to two causes: (i) Organic, including the attacks of fungi particularly *Phytophthora* sp. on the nuts or nut-branches; (ii) Mechanical or physiological, i.e., due to environmental conditions, e.g., the breaking of the fruiting branch due to the removal of the support given by the subtending leaf." In a subsequent paper,[3] he says that the fall may be caused by a water-logged condition of the soil after the heavy rains which cuts off the air supply to the roots, thus interfering with the vital processes of the roots. This may lead to a reduced absorption of water which in effect is equivalent to a water shortage.

The fall of the nuts referred to in this paper is caused by a fungus. Nuts of all sizes varying from small ones to those nearly mature are found to drop down. The fall was fairly common but heavy after the incessant rains of July and August. When the fallen nuts are examined they are found to be of a dark brown or blackish brown colour at the base, the place of attachment (Plate I). These discoloured patches are in some cases confined to the basal region of the nuts alone or in others extend to nearly the basal half of the fruits. They present a water-soaked appearance. Newly fallen nuts show on examination a whitish filmy growth of fungus over the brown patches (Plate I, A). This growth consists of the mycelium and sporangia of the fungus (*Phytophthora* sp.) causing this disease. On breaking open a fallen nut the husk is found soft and rotten in patches where there is external discoloration. The kernel is soft and partly or wholly rotten, depending upon the stage of the disease, emitting a very unpleasant odour and being unfit for consumption (Plate I, B and C). When examined under the microscope it is found fully invaded by the mycelium of the fungus. The

[1] Ashby, S. F. Notes on two diseases of the coconut palm caused by fungi of the genus *Phytophthora*. *West Ind. Bull.*, XVIII, p. 63.

[2] Gadd, C. H. Nut-fall of coconuts. *Ceylon Dept. Agri. Bull.* 53.

[3] Gadd, C. H. A possible physiological cause of nut-fall of coconuts. *Year-Book of Dept. Agri., Ceylon*, 1923.

milk of the nut is brown in colour, giving a fœtid smell. Sometimes the stalks of the fruit and the nut branches may be affected. The main axis of the inflorescence and the individual nut branches turn dark brown and rot (Plate II, 1), with the result that all the nuts on the diseased bunches drop off though the nuts themselves may not have been affected. The hyphæ are found (in sections of the pericarp of young fruits) to be mainly inter-cellular and send in finger-shaped haustoria into the cells.

From the young rotten nuts received, the fungus was brought into culture. Single germinating sporangia from agar plates were picked out by a platinum scoop and transferred to oat agar tubes. They germinate readily in glucose agar in 3 to 4 hours. The fungus grows luxuriantly on oat-juice, corn-meal and French-bean agar cultures and on boiled rice with the production of numerous sporangia. On starch agar the growth is scanty but sporangia are formed. On sterilized bits of young nuts placed in Roux potato tubes there is a good growth of the fungus with copious formation of sporangia. Neither in artificial cultures nor in nature are any oospores noticed. Only sporangia are produced. These measure from 32 — 64 × 27 — 42μ. This fungus resembles *Phytophthora omnivora* var. *Arecæ* in every respect. The morphology of this fungus has been very elaborately dealt with by Dr. Coleman in his Bulletin.[1]

With the fungus from cultures, inoculations were made at Coimbatore on fruits and buds of coconut and arecanut palms. The fruits were placed singly inside moist chambers or hung in bunches inside glass cages. Fruits on the standing coconut trees also were inoculated. Bits of the fungus mycelium and suspensions of sporangia and zoospores were used. The climatic conditions of Coimbatore are unlike those met with on the West Coast. This fungus seems to thrive well only in an atmosphere saturated with moisture after heavy rains as on the West Coast and unlike that at Coimbatore. Yet all attempts were made to create the same atmospheric conditions artificially by the use of damp cotton-wool and spraying the plants with water every morning and evening. The nuts used for infection in the laboratory were sterilized, by first dipping them in mercuric chloride solution (of 1/1000 strength) and then washing them with sterile water two or three times. The buds were enclosed within broad chimneys whose ends were loosely plugged with cotton-wool. A fine spray of sterile water was given to keep the inside of the chimney moist.

[1] Coleman, L. C. Diseases of the areca palm. I, Koleroga. *Mysore Dept. Agri. Bull., Myc. Ser.*, No. 11.

Explanation of Plate II.

1. Parts of the coconut inflorescence showing discoloration.
2. Coconuts inoculated with *Phytophthora omnivora* var. *Areca* showing discoloration on the surface and mass of whitish mycelium

milk of the nut is brown in colour, giving a foetid smell. Sometimes the stalks of the fruit and the nut branches may be affected. The main axis of the inflorescence and the individual nut branches turn dark brown and rot (Plate II, 1), with the result that all the nuts on the diseased branches drop off though the nuts themselves may not have been affected. The hyphæ are found (in sections of the pericarp of young fruits) to be mainly inter-cellular and send in finger-shaped haustoria into the cells.

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EXPLANATION OF PLATE II

[illegible]

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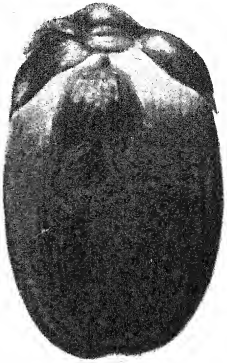
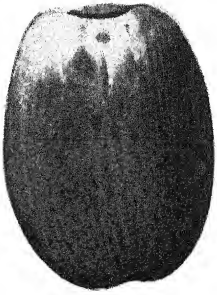
[1] Coleman, L. C. Diseases of the areca palm. I, Koleroga. *Mysore Dept. Agri. Bull., Myc. Ser.*, No. 11





1 2

"MAHALI" DISEASE OF COCONUTS

Inoculation experiments with the fungus Phytophthora omnivora *var.* Arecæ *on fruits, inflorescence and buds of coconut and arecanut palms.*

EXPERIMENT I. Coconut *Phytophthora* on coconut.

No.	Parts inoculated	Date of inoculation	Date of infection	Number inoculated	Number infected	Interval of days	Results of infection	REMARKS
1	Plucked fruits, 3 inches in length.	3-9-22	8-9-22 to 10-9-22	5	5	6	Fruits showed rotting and discoloration—fungus found on the outside.	Nuts plucked from healthy trees—placed in moist chamber with sterilized moist cotton-wool on the inoculated portion to prevent the fungus from drying up.
2	Do.	Control	..	..	..	..	Fruits did not show any discoloration.	....
3	Fruits *in situ* on bunches plucked, 4 inches in length.	25-9-22	1-10-22 to 2-10-22	16	15	6	Fruits discoloured, rotted and dropped from the bunches.	Nuts in 2 bunches, 8 in each—hung inside glass cage—bottom with a layer of sterile moist sand.
4	Do.	Control	..	..	..	..	Fruits unaffected—remained long on the bunches even for a week after the inoculated ones in (3) had dropped down.	....
5	Fruits on standing trees, 3 inches in length.	20-9-22	26-9-22 to 28-9-22	4	4	7	Fruits rotted and dropped down—showed *Phytophthora* fungus on the surface.	Fruits on trees 16 years old and 12 feet high.
6	Do.	Control	..	..	..	..	Fruits unaffected and remained on the bunches.	....

EXPERIMENT I. (*concld.*)

No.	Parts inoculated	Date of inoculation	Date of infection	Number inoculated	Number infected	Interval of days	Results of infection	REMARKS
7	Fruits on standing trees, 4 to 7 inches in length.	25-10-22	1-11-22 to 2-11-22	8	7	7	Fruits discoloured and dropped down, showing *Phytophthora* fungus on the discoloured portions.	Trees 7 years old and 9 feet high.
8	Do.	Control	..	..	..	..	Fruits unaffected and remained on the bunches.	Trees 7 years old and 10 feet high.
9	Stalk of inflorescence on standing tree—wounded.	15-10-22	22-10-22	1	1	8	Stalk of inflorescence rotted at the inoculated portion and hung down and could be easily pulled out.	The stalk was scraped a little and the fungus placed on the wounded portion.
10	Do.	Control	..	..	..	..	Remained healthy.	....
11	Do. unwounded.	15-10-22	..	1	nil	..	No discoloration. Remained quite healthy.	....
12	Do.	Control	..	..	..	..	Remained healthy.	....
13	Folds of the unopened leaf-bud—unwounded.	18-9-22	..	2	nil	..	No discoloration noticed.	Fungus was placed between the folds of the unopened bud and the inoculated portion was enclosed by a chimney and both ends plugged with sterile cotton-wool to keep the inside of the chimney in a moist condition.
14	Do.	Control	..	..	..	..	Do.	....
15	Do. wounded.	10-10-22	22-10-22	2	2	12	Infection localised—small discoloured patches round the inoculated region.	Leaf wounded and inoculated.
16	Do.	Control	..	..	..	..	No discoloration.	....

EXPERIMENT II. Coconut *Phytophthora* on arecanut.

No.	Parts inoculated	Date of inoculation	Date of infection	Number inoculated	Number infected	Interval of days	Results of infection	REMARKS
1	Arecanut fruits on a bunch plucked, 150 fruits.	10-11-22	15-11-22	Whole bunch of 150	Whole bunch	5	All the fruits rotted, dropped down and showed the fungus on the surface.	Bunch was plucked from a healthy tree—hung inside glass cage — bottom with a layer of moist sterile sand. Cultures of the fungus with sporangia and zoospores were sprayed on the bunch.
2	Do. 1 bunch.	Control	..	..	..	..	All the fruits remained healthy on the bunch.	....
3	Arecanut bud unwounded.	15-10-22	20-10-22	3	3	5	Buds discoloured and rotted	Fungus material with sporangia and zoospores was poured between the folds of the bud—buds enclosed by a chimney.
4	Do.	Control	..	..	..	..	No discoloration ..	....
5	Trunk—soft portion below the crown—wounded.	25-9-22	4-10-22	1	1	10	Whole crown rotted, hung down, showing copious mycelium and sporangia.	The outer leaf-sheath at the base of the crown was bored and the fungus material was put inside the bore.
6	Do.	Control	..	..	..	..	Remained healthy ..	....

EXPERIMENT III. Arecanut *Phytophthora* on coconut.

No.	Parts inoculated	Date of inoculation	Date of infection	Number inoculated	Number infected	Interval of days	Results of infection	REMARKS
1	Plucked fruits, 3 inches in length.	10-11-22	17-11-22	4	4	7	Fruits showed discoloured areas. Fungus found on the surface.	Fruits kept inside moist chambers and moist cotton-wool placed on the inoculated portion.
2	Do.	Control	..	..	..	..	No discoloration	....
3	Fruits on standing trees, 4 inches in length.	6-11-22	12-11-22 to 13-11-22	4	4	7	Fruits discoloured and dropped down.	Trees 7 years old and 9 feet high.
4	Do.	Control	..	..	..	..	No discoloration. Fruits remained on trees.	....

Results of inoculations.

(1) *Coconut Phytophthora on coconut.*

Coconut fruits inoculated with the fungus developed small brown spots at the place of inoculation in six to seven days after the date of inoculation (Plate II, 2). These spots gradually increased in size until the whole of the basal region assumed a dark brown colour. A whitish growth of the fungus with a copious formation of sporangia appeared over the discoloured areas. On cutting open the nuts the pericarp was found to be soft and rotting. Sections of the pericarp showed the presence of the inter-cellular mycelium with the fingershaped haustoria projecting inside the cells. The fungus infects the young fruits readily without wounding. But a fully saturated atmosphere seems to be absolutely necessary, since one set of inoculations conducted when the climatic conditions were dry was not successful in spite of using moist cotton-wool. The fungus, so far, is known to be confined to the fruiting bunches and not affecting the bud. Buds which were inoculated after wounding showed only small localised spots while those which were not wounded did not take infection.

(2) *Coconut Phytophthora on arecanut.*

Arecanut buds and fruits inoculated with the fungus isolated from coconuts readily take the infection. The nuts rot and fall off the bunches. As noticed in the "Mahali" disease of arecanuts, a greyish white coating of the fungus appeared on the fruits. Thus from the results of the inoculations, it is seen that the fungus causing the "Mahali" or nut-fall of coconuts is the same as the one responsible for the "Mahali" disease of arecanuts.

(3) *Arecanut Phytophthora on coconuts.*

Arecanuts affected by "Mahali" were obtained and the fungus *Phytophthora omnivora* var. *Arecæ* isolated from them. Four coconuts about three inches long were inoculated with this fungus, and kept inside moist chambers with sterile moist cotton-wool over them. In six to eight days brown patches formed on the fruits round the inoculated places and these gradually increased in size. The control was not affected. No spot formation was noticed in any of these. Experiments tried on standing coconut trees showed also positive results.

So far this disease has been noticed only in a few localities, though it cannot be said with certainty that it is confined to the places shown in the

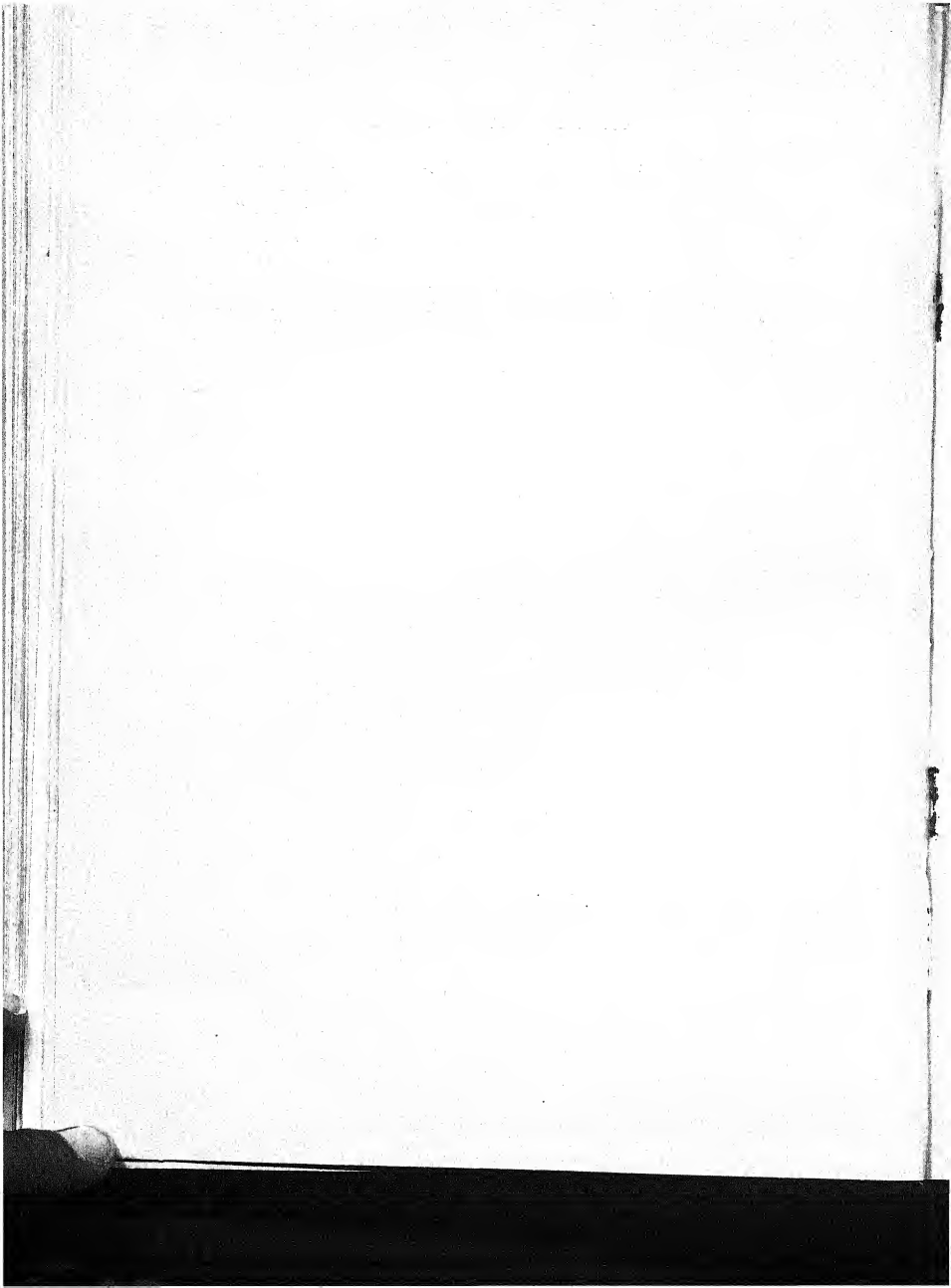
map. From the few reports received, the nut-fall appears to be not very serious and shows itself only in gardens where the two palms (arecanut and coconut) are grown together and where the "Mahali" disease on the former has been severe. The "Mahali" of arecanuts has been prevalent in Malabar for several years and the appearance of the disease on coconuts growing in arecanut gardens affected by "Mahali" clearly shows that the source of the disease on coconuts must obviously be from arecanuts. From the arecanuts to the coconuts, when the favourable conditions prevail, is but an easy step. When the arecanut palms in a garden are sprayed sufficiently early and "Mahali" is prevented, nut-fall in coconuts does not appear in that garden. In one garden in Mullesseri, South Malabar, there was a severe outbreak of the disease on arecanuts last year. This spread to several coconut palms and there was nut-fall. But during this year the owner sprayed the arecanuts early in the season and thus prevented the appearance of the disease. No disease has appeared on the coconuts also.

The control measures adopted to check the "Mahali" disease of arecanuts can well be employed as effectively in the case of the coconut disease also. Before commencing to spray, all the diseased and rotten nuts should be picked up and burnt along with the rotten and decayed stalks and inflorescence branches collected from the tree. The bunches should be sprayed just before the rains set in and once again at interval of clear weather. This may be done when the arecanuts are being sprayed. Every coconut palm growing in a group of arecanuts in an infected area should be sprayed with Bordeaux mixture as a preventive. This is the only method of combating the disease. The cost of spraying the bunches on a coconut palm is twice that of spraying the bunches of an arecanut, i.e., it comes to about Rs. 2-4-0 for 100 trees or about 5 pies per tree. One nut saved will meet this cost. On an average the income from each coconut tree is about Rs. 2 per annum.

Summary.

A disease of coconuts called nut-fall or "Mahali" has been noticed in certain parts of Malabar after the heavy south-west monsoon during the last two years. Young as well as nearly mature nuts fall off in large numbers. The basal portions of the fallen nuts are of a dark brown colour with a growth of the fungus *Phytophthora* sp. over them. The kernel of the affected fruits is rotten and useless. Sometimes the inflorescence axis and branches are affected.

Inoculation experiments show that the *Phytophthora* sp. is the cause of the disease. Cross-inoculations prove that this fungus is the same as the one—*Phytophthora omnivora* var. *Arecæ*—which causes the "Mahali" or "Koleroga" of arecanuts. The disease appears on coconuts only in gardens where they are grown interspersed with arecanuts and where the latter are affected by "Koleroga," and coconuts get the disease from arecanuts. Spraying with Bordeaux mixture was recommended as a remedial measure. It has been found successful in preventing nut-fall wherever tried.



CONTENTS

THE ERADICATION OF *CYPERUS ROTUNDUS* L.

(A STUDY IN PURE AND APPLIED BOTANY).

BY

S. B. RANADE, B.A., M.Sc. (Bom.)
(*Under the direction of a Research Committee of the Bombay Department of Agriculture*).

ARRANGED AND WRITTEN

BY

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I. INTRODUCTION.

"THE contributions of scientific research to agricultural development in the past have been enormous. In fact the entire structure of modern agriculture is founded on scientific discoveries. It will, however, only require a hasty and superficial survey of the situation to indicate that the opportunities for still further contribution are even greater at this time than they have ever been in the past. Certain fields of agricultural research have been almost entirely neglected up to the present time. Probably the most outstanding one is the weed problem. Weeds undoubtedly do as much to reduce the annual crop as do insect pests or plant diseases and yet they are just beginning to receive attention."[1]

The research described in this memoir has been done under auspices and in a manner which themselves deserve a short description.

[1] *Research Work of the Department of Agriculture*, by E. D. Ball, Director of Scientific Work, U. S. Department of Agriculture, in the *Chemical Age*, article copied in full in the *American Fertilizer*, Jan. 26th, 1924, pp. 62–68.

The Trustees of the Sassoon David Trust Fund, Bombay, have from time to time made grants for research work in connection with agricultural development. In the present instance, they supplied the means "for the investigation of the eradication of the most serious weeds of cultivation, and especially of the *Lavala* weed," this purpose being so outlined by the Director of Agriculture, Dr. H. H. Mann, in making his proposals.

It was early decided to concentrate on the lavala weed, *Cyperus rotundus*. All writers agree as to the noxious character of this weed. Cooke[1] says, "A very troublesome weed of cultivation, eradicated with difficulty owing to the stolons becoming woody." Roxburgh[2] states, "This is by far the most common species we have in India; it delights in a moist sandy soil though it grows abundantly everywhere. It is by far the most troublesome weed we have in our gardens, there is no extirpating it as every little bit of the root grows readily." This character is confirmed by a remark in a recent American publication,[3] that "nothing serves so well to propagate it as to plough and re-plough with a view to destroy it."

In a letter to the Sassoon David Trust Fund Trustees in 1920 the then Director of Agriculture of the Bombay Presidency, Mr. Keatinge, said, "The problem of eradication, particularly in some of our most fertile areas like Kaira and Gujarat, is an unsolved problem, and the lavala weed itself probably reduces in these very fertile tracts annually the yield by 25 to 30 per cent."

The plant is widely distributed in all warm regions. It is impossible to locate the centre of that distribution. It is found in Australia, America, Africa, and the warmer parts of S. Europe, and, in fact, anywhere in the tropics, sub-tropics and adjacent regions.

Its main peculiarity and its main strength are the tubers formed on its underground wiry rhizomes, by means of which it stores up food and propagates itself.

The organization of research in general and agricultural research in particular is the subject of considerable discussion and trial in most countries at the present moment. In this, as in several other of our recent departmental researches, we have followed a method which has proved efficient and which seems to have found favour elsewhere also. This was to control the research by a committee in which various interests were represented and with a chairman whose own technical and administrative training would enable him to

[1] Cooke, J. *Flora of the Bombay Presidency*, II, 872, 1908.
[2] Roxburgh, W. *Flora Indica*, 66, 1874.
[3] Georgia, Ada. *Manual of Weeds*, 68, 1914.

guide the actual worker or workers. The committee appointed by the Director of Agriculture consisted of—

The Economic Botanist to the Government of Bombay (Chairman)	Dr. W. Burns.
The Professor of Agriculture, Poona College of Agriculture	Prof. J. B. Knight,
and later	Prof. B. S. Patel.
The Deputy Director of Agriculture, Gujarat ..	Rao Saheb Bhimbhai Desai.

For the actual conduct of the research Mr. S. B. Ranade, B.A., M.Sc. (Bom.) Bombay, was selected. His work was planned by the committee and carried out under the personal supervision and with the assistance of Dr. W. Burns. The laboratory work was done in the laboratory of the Economic Botanist, College of Agriculture, Poona, and the field work on various farms of the Bombay Department of Agriculture, on certain privately owned fields and in the Empress Gardens, Poona.

The object of this research was to find means of eradicating the lavala weed. This is not the first time that departments in India and elsewhere have addressed themselves to this problem. Their method of attack has usually been experiment on a field scale with agricultural implements.

After careful consideration the committee came to the conclusion that there was really only one sound way of dealing with this problem, namely, to study the lavala weed itself first, and get to know all that one could about its life cycle, and then to attack it at the weakest point of that life cycle.

It was recognised that this meant in the beginning a botanical enquiry with possibly slow progress, but that the foundation of sound scientific knowledge so laid would bear the weight of any superstructure of agricultural practice that might be built up as the result of that work.

The results have entirely justified the method of controlling the research, the choice of the actual worker, and the decision to study first the plant and only later to deal with field practice.

The whole research is here described.

II. The Botany of *Cyperus rotundus*, L.

1. *Systematic Position.*

The natural order *Cyperaceæ* is a well-defined family, superficially similar to grasses but differing actually in many essential points, both botanically and agriculturally. It is not essential in this Memoir to go into these

details, which are available in any text-book. The genus *Cyperus* itself belongs to the sub-family of the *Scirpoideæ-Cyperineæ* and numbers about 400 species, mainly found in the tropical and sub-tropical regions of the earth. In South England there are two species, rare in occurrence.

Cyperus rotundus is cosmopolitan within the limits of distribution of the genus and is, in all the countries where it occurs, a formidable weed of cultivation. Possibly for this very reason it has not received that detailed study at the hands of botanists which rarer plants enjoy. In the various floras of India the plant naturally finds a place and we quote here Hooker's[1] description.

"India, alt. 0-6,000 ft., a pestiferous weed. Distrib. All warm regions. Glabrous. Stolons slender, up to 4-8 in., hardening into wiry roots, thickened into black woody ovoid tubers $\frac{1}{3}$-1 in. in diam., not (or very obscurely) zoned. Stems subsolitary, 4-32 in. at top triquetrous. Leaves long, often overtopping stem, $\frac{1}{6}$-$\frac{1}{3}$ in. broad. Umbel frequently compound, primary rays 2-8 in., spikes loosely spicate of 3-8 spikelets ; but umbel sometimes large, sometime reduced to 1 head and (in a common Calcutta form) to 1 spikelet." The plant is listed by Clarke[2] but not figured.

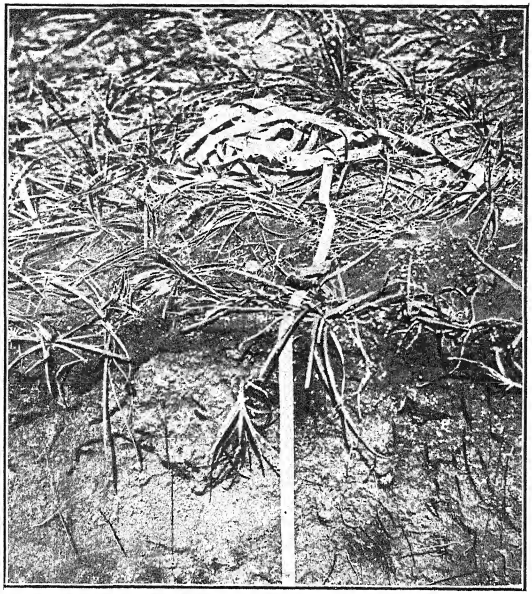
2. *Common Characters and Names.*

Cyperus rotundus is easily recognizable. Its leaves spring directly from the ground without any apparent stem. They are of a peculiar bright green and are marked by a deep furrow in the middle. When full grown they sprawl about on the surface of the soil. The plant thus has a characteristically untidy habit. (Plate I, 1.) From the centre of the rosette of leaves springs up the grass-like inflorescence. A plant so common, so detrimental to agriculture and so recognizable was bound to receive common names. In the Bombay Presidency its vernacular names are *Lavala* and *Nagarmotha* (in Marathi), *Gundardo* (in Gujarati), and *Korai Tek* (in Kanarese). The Tamil name is *Kizhangu*. Throughout this memoir, when referring to the plant by its common name, we shall call it Lavala.

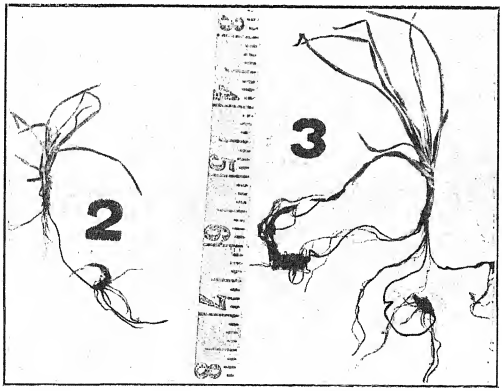
3. *Varieties.*

In Gujarat two forms of the plant are distinguished by the people and named Chido and Chidi. In Chido vegetative growth above ground is vigorous and the leaves are long and broad. Below ground the tuber formation is also vigorous, the tubers are long and broad, formed at large

[1] Hooker, J. D. *Flora of British India*, VI. 615.
[2] Clarke, C. B. *Botanical Sub-subareas of British India*, 36.



1. The Lavala plant above and below ground.



2. The Lavala seedling.

intervals on a deeply penetrating rhizome system. Most of these tubers do not sprout but remain as reservoirs of food and water. In Chidi the shoots are stunted and bushy, and the leaves small. The tubers are small and more superficially placed on a less extended rhizome system. As a matter of fact, most of the tubers are within a foot of the surface of the soil. These sprout readily and form a dense mass of shoots. It is believed that Chido turns into Chidi and that the latter is the more difficult to eradicate.

We found Chido on moist loose *goradu* (sand and clay) soil and in irrigated fields of black soil. Chidi was found in hard drying *goradu* soil, and also in water-logged or packed black soil. This would seem to indicate that Chidi was a response to unfavourable soil conditions. To test this, tubers of characteristic Chido and Chidi plants were dug up from various places in Gujarat and brought to Poona where they were grown in pots in various soils and conditions. It was found that the formation of the Chido and Chidi forms was absolutely dependent on the soil and water conditions, tubers from either Chido or Chidi giving the opposite form in their progeny according to the environment.

While the variation just mentioned is an effect of the environment and not inherited, there are variations as yet little studied which appear to be inherited. It has been our experience and also the experience of others that any wild species in India, if critically studied, yields sub-species of considerable range of variation. Hooker[1] has in the case of many of the grasses mentioned such. In the case of lavala, the floras give the colour of the glumes as red-brown. We have in addition observed the following colour variations in the glumes, (1) yellowish white, (2) light red, (3) coppery red with a metallic lustre, (4) dark red with a blackish tinge. This colour variation has nothing to do with age, as the colour was determined at the same stage in each case, namely, the time when the stigmas were protruding. In 1921, experiments were made to determine if these colour variations were inherited through tuber propagation. It was found that this was so in all cases, with the curious modification of loss of metallic lustre in the third variety mentioned. We have not been able to pursue these experiments further but there is obviously here a fertile study for the geneticist.

It is noteworthy that Sedgwick[2], a very accurate observer, mentions these colour variations, and even goes the length of correlating them with soil variations. We are unable to confirm such correlation.

[1] Hooker, J. D. *Flora of British India*, VII, 192–193, *Andropogon monticola*.

[2] Sedgwick, L. J. The Cyperaceæ of the Bombay Presidency, *Bom. Nat. Hist. Soc. Jl.*, XXV, No. 4. p. 696, June 1918.

4. *The Seedling Stage and First Tuber.*

From the standpoints of both botany and agriculture, the tuber-forming character of this plant is of the greatest importance. A complete understanding of the nature and origin of these tubers demands a study of the plant from its seedling stage onwards. We shall now record our observations.

The visible "seed" is really a fruit, being a triangular compressed hard-coated and brown nut of minute size (1/30th by 1/40th inch). Within this is the true seed, containing endosperm and a microscopic embryo. On germination this embryo produces a seedling of the type well-described by

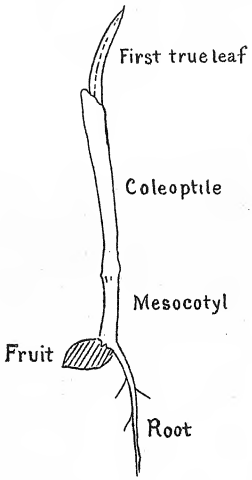


FIG. 1. Seedling of *Cyperus rotundus* L.

Goebel[1]. Figure 1 shows diagrammatically the arrangement of its parts. Within the seed is the haustorial portion of the cotyledon, whose business it is to transfer to the shoot the stored material of the endosperm. Following it is a curious elongated body, the mesocotyl, which connects with the tubular sheath (coleoptile). All three parts (haustorial part of the cotyledon, mesocotyl and coleoptile) are regarded by Goebel as

[1] Goebel, K. *Organography of Plants*, II, 413, 1905.

collectively the cotyledon. Where the mesocotyl joins the coleoptile is the point of origin (within the coleoptile) of the leaves. In other words this is the site of the terminal bud. The mesocotyl is explained biologically as a means for facilitating boring through the earth by the coleoptile with its contained stem-bud. This adaptation is in itself a foreshadowing of the geophilous habit of which we shall have more to say later. The amount of elongation of the mesocotyl is to some extent determined by the depth of earth above the fruit. The radicle elongates and develops a few secondary roots. At this stage occasionally the fruit-coat shrivels, leaving the endosperm protected by the yellow seed-coat only. The junction of the mesocotyl and the coleoptile swells and adventitious roots start therefrom, the coleoptile itself bursts and the first true leaf emerges. After the development of two or three leaves and the growth of several adventitious roots the radicle darkens and shrivels. The contents of the seed remain attached to the seedling for a considerable time.

From the swollen base of the shoot (i.e., the swollen junction of mesocotyl and coleoptile), which we shall call the *basal bulb*, now appear one or more positively geotropic shoots which elongate and each forms a tuber at its end. (Plate I, 2.) These tubers launch out into vegetative growth of their own, but we shall leave their development for the present and concentrate our attention on the life history of the seedling thus far revealed.

5. *The Geophilous Habit.*

The salient point of this life history is the haste of the plant to form a tuber. This first tuber formation is completed within ten to fifteen days after germination. As an interpretation of this we cannot do better than refer to Miss E. Sargant's[1] brilliant paper "*A Theory of the Origin of the Monocotyledons founded on the Structure of their Seedlings*" and especially to that part of the paper which deals with the development of the geophilous habit. Miss Sargant deals specially with the correlation of the tuber-forming habit with the concrescence of the two cotyledons of the dicotyledon into the one of the monocotyledon, but from our point of view (that of mere adaptation to environment) her remarks are equally illuminating and we make no apology for quoting some of them.

"The formation of underground root-stocks, of tubers, corms, and bulbs, is characteristic of the plants called 'geophilous' by Professor Areschoug. The general definition of the term which he gives in part I

[1] Sargant, Ethel. A Theory of the Origin of Monocotyledons founded on the Structure of their Seedlings. *Ann. Bot.*, XVII, I, 1903.

is very wide; 'We include under that head such plants as form the buds by which they reproduce the shoot underground; those plants in fact which develop their aerial organs more or less completely beneath the surface of the soil.' Defined in this way the term would include all biennials and herbaceous perennials of the temperate and arctic zones, for the aerial shoots of all such plants disappear during the winter, and are replaced in the following spring by the development of buds formed underground....

"In order to use the short season of vegetation to the best advantage the geophilous plant or geophyte must be furnished with a store of nourishment, and this is placed at some distance below the surface of the soil for protection against the cold or heat of the dead season. A plant so provided can throw up leaves and flowers at a few days' notice from the bud attached to its swollen axis or tuberous root.

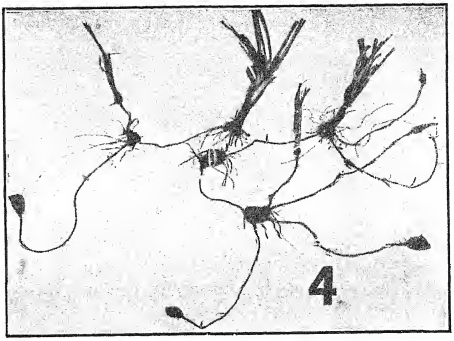
"The leaves when once above the ground make the most of their short life. They restock the underground organs with food for the following season, and they support the flowers, and later the maturing fruit, until the seed is ripe. When this occurs before the advent of the cold or drought withers the aerial shoots, the cycle of development is complete, but in such localities it must often happen that an early frost or a dry season kills all the seed formed by a plant before it is ripe.....

"When we consider the conditions under which a typical geophyte lives, it is very clear that its seedlings must be even more perfectly adapted to the environment than the mature plant in order to have a chance of surviving.

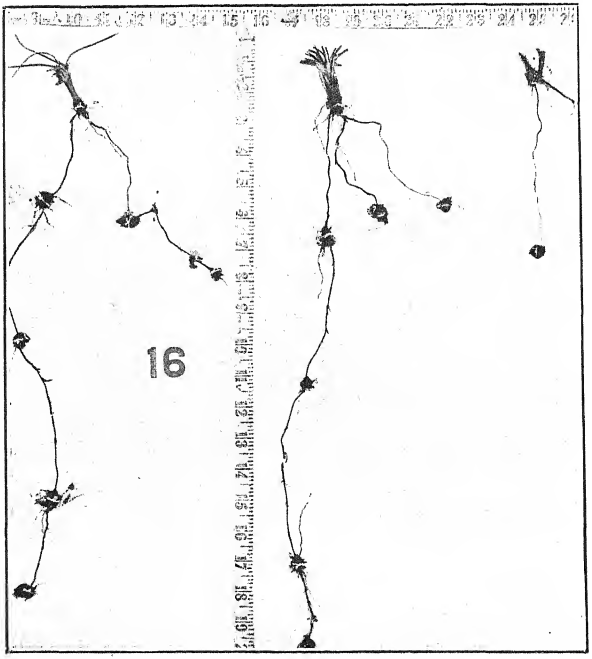
"The seed formed at the end of the growing period is commonly capable of resisting a considerable degree of cold or drought in the long dead season. When the genial weather returns and it germinates, the seed is confronted with a difficult problem. During the short period of vegetation the growth of the seedling must proceed in such a way that the structure completed by the end of the season is capable of living through the severe weather which follows.

"Accordingly we find that the seedling begins at once to form its underground organs.....

"In all these cases, however, the production of assimilating surfaces seems to be an object of secondary importance to the seedling of a geophilous plant in its first season. The formation of adequate subterranean organs at a safe distance below the surface of the soil is the condition on which the life of such a seedling ultimately depends, and its powers are devoted in the first place to this task."



1. Tubers giving rise to deeper tubers.



2. Typical droppers.

6. *Other Tuber-forming Species.*

Cyperus rotundus is not the only tuber-forming species. There are also *C. tuberosus*, and *C. esculentus* in the Bombay Presidency, and (so far as one can judge from plates and descriptions) *C. stoloniferus* elsewhere. *C. bulbosus* is not quite in the same class, since its tuber is more of the nature of a rosette of fleshy leaves. Of all the tuber-forming species, however, *C. rotundus* is easily first as to the depth and extent of its subterranean tuber-system, the beginning of which we have just been describing. It is, therefore, a plant which has developed the maximum amount of protection against drought, heat or cultivation. Little wonder that it has become one of the main weeds of the tropics and sub-tropics.

7. *General Morphology and Physiology of the Tubers.*

The tuber appears always to be the condensed end of an underground shoot. It consists of several short internodes, much swollen and bearing between them the scale leaves and buds of the nodes. The tuber is ovoid in shape and at its narrower end bears a terminal bud. The scale leaves of all the buds are deciduous. The tuber is at first externally white, but changes colour with age through red and brown to black. Each bud of a tuber can produce a rhizomatous shoot which either forms a tuber at its tip (and so on to form a chain of tubers with intercalated rhizomes) or else produces a rhizome that ends in an aerial shoot. The dormant apical bud can form only the latter.

The tubers are storehouses of food-material, mainly starch, and in addition are perennating organs buried at varying depths, admirably adapted to ensure the survival of at least some of their number.

We shall have, later in this memoir, to discuss at some length the formation and germination of these tubers, but we may for the present describe generally their behaviour.

After a plant has formed its first tuber it may form further rhizomes from a swelling (at the junction of the mesocotyl and coleoptile, henceforth to be called the *basal bulb*) and also from the tuber. There are two chief types of rhizomes those which are negatively and those which are positively geotropic. The positively geotropic shoots seek deeper layers and there usually form another tuber from which still another shoot going downwards may be formed. (Plate II, 1.) We thus find chains of tubers descending to considerable depths. We have provisionally called a rhizome which descends to some depth a "*dropper*". (Plates II, 2 and III, 1.) Any tuber whether on a dropper or not may form a negatively geotropic shoot which strives to reach

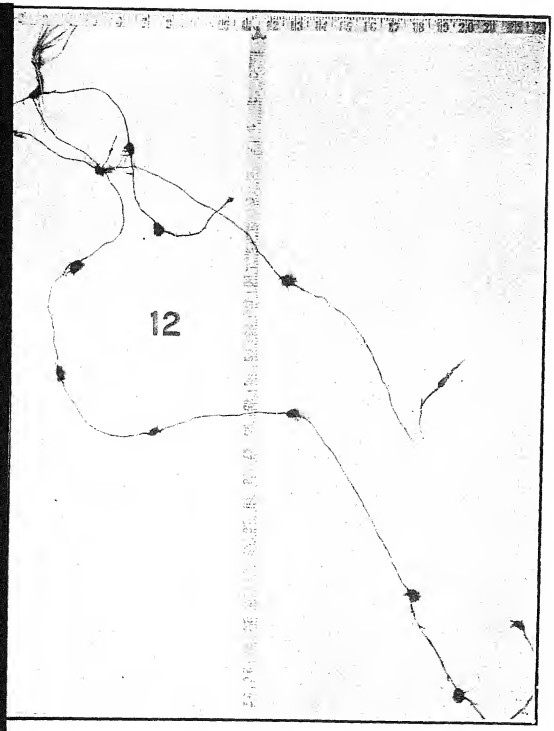
the light. If the tuber which forms this type of shoot is at a great depth then the shoot may have a considerable struggle to get to the surface and may possibly fail. In certain cases the plant resorts to the device of forming advanced bases in the form of intercalary tubers on the way up, into which it presumably sends its food material and so gets a fresh start from a higher level (Plate III, 2). In addition to rhizomes with a pronounced positive or negative geotropism there are also rhizomes which are more or less diageotropic and which spread in a horizontal direction either ending in tubers within the same soil layer, or eventually bending up or down.

The result of all these developments is the production underground of a large number of tubers at different depths, connected by rhizomes, and with certain rhizomes ascending to the surface and there producing the leaves which feed the tubers with starch. The number of shoots above ground is no indication of the number of tubers below ground.

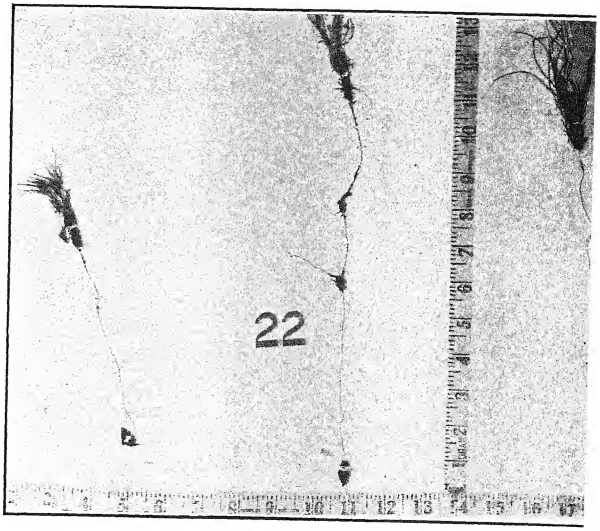
Put in another way this means that only a few of the tubers are actually in a state of growth. They are serving as food stores or exchanges but, unless some special stimulus is applied, they will not further burst into growth. Such a stimulus is the isolation of the tuber. In the various experiments recorded in detail later on, the effects of such planting of isolated and partially connected tubers are given.

At present we may draw attention only to one or two points in the germination of the tuber which are of interest. The isolated tuber, like the seed, makes its first business the establishment of an aerial connection. To this end, from one or more of its buds, it sends up a shoot which grows vertically and has considerable power of penetration even in hard soil. The apical bud is covered by white tough pointed scales forming a spearhead. On this bud reaching the surface the rhizome ceases to grow in length, the scale leaves which are exposed turn green and, instead of scale leaves, normal leaves are now developed. There is no further noticeable elongation of the axis until the formation of the inflorescence. At the junction of the normal leaves and the rhizome is formed a swelling apparently comparable to that which forms between the mesocotyl and the sheath of the seedling and from which, as in the seedling, shoots and roots may develop. We shall use the term *basal bulb* for this swelling also (Plate VI, 2). From the other buds of the tuber, after the establishment of the aerial connection, any kind of shoot may be formed, and, from the nodes of the tuber, roots may also develop.

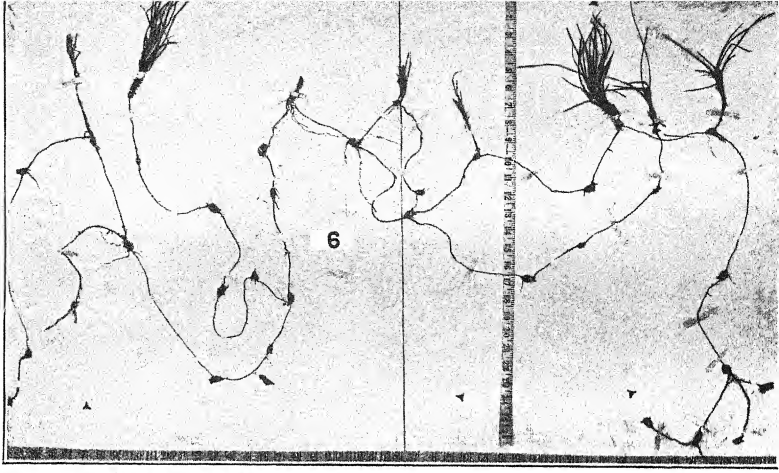
In superficially placed tubers most of the buds may develop to form shoots. In deeply placed tubers usually only one makes the attempt, the



1. Very long "droppers."



2. Growth of deeply planted tubers.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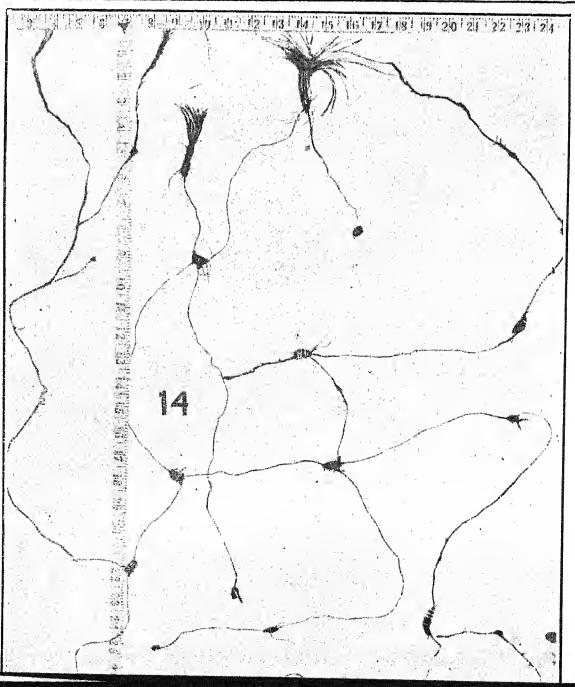
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tuber apparently concentrating all its powers in making one supreme attempt to get to the surface and not wasting material on several ascending organs.

Tubers may be cut or scraped without damage to their germinating powers.

We hope that the above details suffice to give a working idea of the structure of this plant, which is in reality a colony of tubers connected by rhizomes, distributed through a considerable depth of soil and fed by a comparatively small number of aerial shoots (Plate IV). Any tuber and, in fact, any bud of any tuber is a potential colony, and suffers not in the least by severance from its parent. The difficulties of eradicating such a plant already suggest themselves only too clearly.

8. *General Ecology of* Cyperus rotundus *L.*

Cyperus rotundus is essentially a weed of cultivation, and is specially to be found in irrigated fields. Outside cultivated areas it is rare, and seems to be unable to stand up to the competition of other vegetation. This characteristic is utilised by the Gujarat farmers who occasionally allow a very badly infested field to go fallow for several years continuously. The other vegetation (mainly grass in the later years) ousts the lavala completely.

We have not had the time nor the opportunity to study the various stages in such a succession, but this is obviously a most interesting subject of research. We mention later some experiments on the effect of grass on lavala. Lavala does not grow in very salt land or in land which is inundated. On really salt land it is replaced by *Cyperus lævigatus* and on really marshy land by other Cyperaceæ. Its drought-resisting powers are considerable, but we consider these as more due to the disposition of its tubers than to any special xerophytic adaptations.

The lavala plant in the Bombay Presidency flowers in July and August, (after the heavy rains of the first part of the monsoon), again in October and again in February and March. These are the times of general flowering, but flowering may occur at any time.

9. *The Anatomy of* Cyperus rotundus *L.*

Plowman[1] has dealt fully with the detailed anatomy of the whole order (Cyperaceæ), and there is little left to be described. Sabnis[2] gives anatomical

[1] Plowman, A. B. The Comparative Anatomy and Phylogeny of the Cyperaceæ. *Ann. Bot* XX, 1906.

[2] Sabnis, T. S. The Physiological Anatomy of the Plants of the Indian Desert. *Jour. of Ind. Bot.*, II, pp. 167—173, 1921.

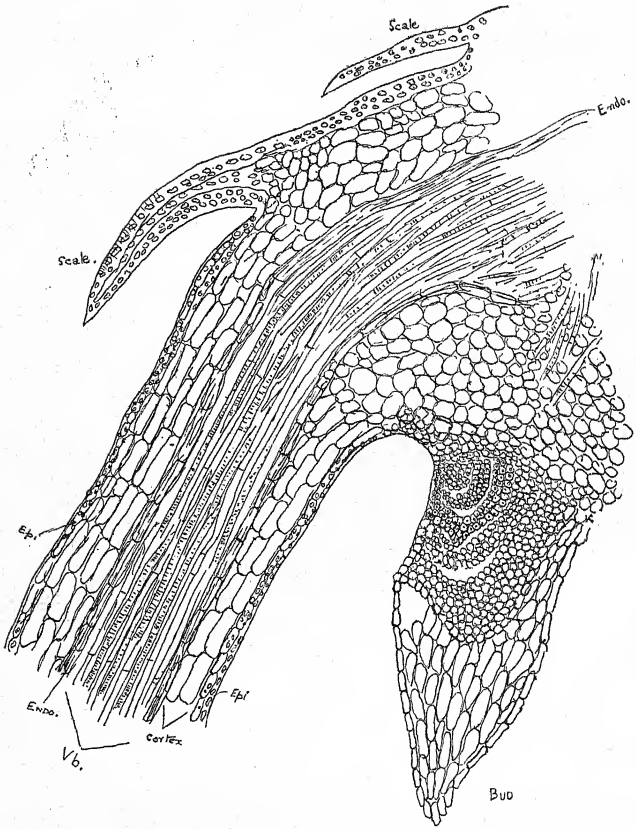


Fig. 2. Longitudinal Section of Rhizome and Tuber.
Vb=Vascular bundle.
Ep.=Epidermis.
Endo.=Endodermis.

descriptions and illustrations. From our point of view the most important organ for anatomical study is the tuber.

Tuber Anatomy :—

Fig. 2 shows the junction of a rhizome and a tuber. The rhizome is bounded externally by a much thickened epidermis. Within this is a starch containing cortex and a lignified endodermis. In old and wiry rhizomes the endodermis is the outer layer, the cortex and epidermis having disappeared. The endodermis is yellow in colour and contains a semi-viscid yellow fluid which does not give the reactions of tannin or suberin. The vascular bundles are normal and close set.

The epidermis of the tuber is slightly sclerenchymatous and may be of more than one layer. Arising from it and the immediately underlying tissues

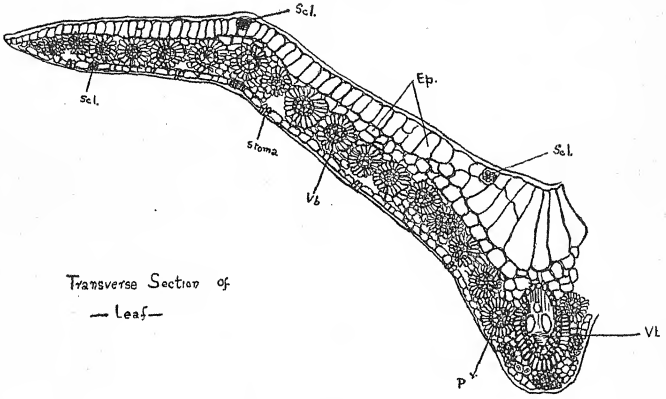


FIG. 3.
Vb=Vascular bundle. Scl.=Sclerenchyma.
Ep.=Epidermis.
P=Palisade.

are the scale leaves. The bulk of the body of the tuber is a large-celled cortex and ground tissue containing starch in excess. The ground tissue

between the vascular bundles narrows down as the stele approaches the rhizome and is continued in the rhizome as elongated thin walled cells between the xylem vessels. The endodermis is continuous throughout tuber and rhizome. Roots originate from a rhizogenous procambium just within the endodermis. In the axil of each scale leaf is a bud and figure 2 shows one of these in section in a growing condition with a branch of the vascular system connecting it up with the main system of the tuber.

Scattered among the starch-containing cells of the ground tissue are isolated cells containing granular protoplasmic sacs. Small refractive granules in these sacs are dissolved in ether. The substance appears to be of the nature of an essential oil or a resin.

In cuts on the surface of tubers we find a copious exudation of gum, accompanied with degeneration of starch in the cells abutting on the cut.

Tubers exposed to the sun and dried have almost empty cells in the cortex and ground tissue. Such starch grains as are found are yellowish in colour and reduced in size.

Tubers exposed for a long time to the sun or exhausted by continued removal of the aerial shoots are reduced to the sclerenchymatous endodermis and the lignified elements of the stele.

Leaf Anatomy :—

The leaf has a strikingly large-celled cutinised upper epidermis which probably functions as a water storage tissue. The lower epidermis is insignificant. There are no stomata in the upper epidermis. On the lower epidermis the stomata are found between the sclerenchymatous sheaths that accompany the vascular bundles. The stomata are present in the density of from 140 to 370 per square millimeter in different parts of the leaf. The assimilating tissue is that round the vascular bundles. In other words the bundle sheaths are also the assimilating cells.

The leaf is on the whole of a xerophytic type.

Rachis of Inflorescence.

Fig. 4 shows this in cross section. There is again a markedly cutinised epidermis. The superficial vascular bundles have an assimilating bundle sheath like those in the leaves. The deeper lying bundles are larger and of normal structure with sclerenchymatous sheaths. The ground tissue is parenchymatous.

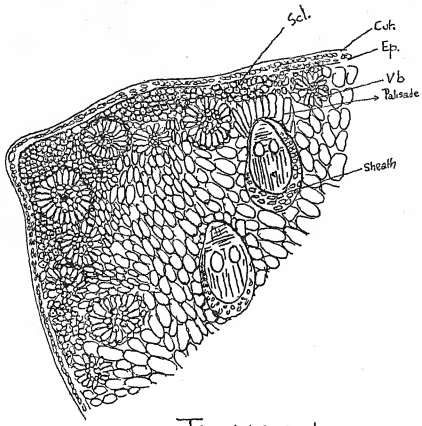


Fig. 4.
Cut.=Cuticle.
Ep.=Epidermis.
Scl.=Sclerenchyma.

III. Experiments in the propagation of *Cyperus rotundus* by seed.

It was necessary to find out as accurately as possible how effectively *Cyperus rotundus* propagates itself by means of seed. In December 1921 the number of seeds produced per inflorescence was determined for one hundred inflorescences. One cannot with ease determine the number of seeds *per plant* because, as we have seen, a plant is a colony of varying size and of limits difficult to define. The numbers found were as follows :—

Class	Frequency
No. of seeds per inflorescence	
1—100	16
101—200	39
201—300	23
301—400	14
401—500	6
501—600	1
601—700	1

The mean number of seeds per inflorescence is 220. The nature of the distribution is that of a skew frequency curve with the mode near the lower limit. The wide range of variation (actually from 10 to 607 seeds per inflorescence) is presumably an indication of the wide range of conditions in which the plants lived.

Taking fifty inflorescences per square yard (a quite moderate figure in an infested field) this would mean the production of 11,000 seeds per square yard or 53,900,000 seeds per acre, and that probably three times in the year. If all these seeds are viable, we have here a very effective means of propagation.

The first tests of viability were made in 1920. The seeds were placed in porcelain dishes on damp sand in a germinating box. From June 1920 till February 1921 these tests were repeated, but no germination resulted. We have not been able to determine the reason for this failure to germinate, unless it be that the seeds were not in contact with the sand, but only with the moist porcelain.

The next tests were made in April 1921 with seeds collected in October-December 1920. These seeds were variously treated before being put to germinate. The treatments given being—

1. Control ... no treatment.
2. Exposure daily to the sun for a month.
3. Heating for three hours continuously at 124°F. (51°C.) in an oven, and cooling slowly.
4. Heating as in (3) and cooling rapidly.
5. Soaking the seeds in hot hydrochloric acid of ten per cent. strength for half an hour.
6. Puncturing the seed coat by a needle.

Fifty seeds were treated in each batch and were then put to germinate, twenty-five (of each fifty) in pots in the open and twenty-five in wet sand in petri dishes in a moist chamber in the laboratory. The seeds in the pots were sown one inch below the surface and the soil was watered daily. The air shade temperature maximum was 94°F. (34°C.) and the moist chamber temperature maximum 84°F. (29°C.). In the pots there was no germination visible in ten days (April 1st to 10th), but in the petri dishes germination occurred as follows:—

Treatment	Germination
1	5
2	8
3	18
4	10
5	8
6	8

TABLE I.

Germination of seeds of Lavala.

Experiment to determine the effect of heat and rest. Heating was done in an oven at 139°F. (95°C.) for one hour.

No. of seeds in each test, 25.

Time :—12th to 30th April, 1921.

No.	Process	In pots—soil 3″ deep, seeds 1″ below the surface. Watered daily	In moist chamber in petri dishes on moist sand		Remarks
			Germinated seeds	Percentage	
I.	*Fresh seeds*—	None of these			Seeds germinated in the conditions of the moist chamber. Heating apparently helps germination.
	1. Unheated..	germinated	6	24	
	2. Heated ..	though watered	16	64	
II.	*Rest of one month*—	daily, till the			
	1. Unheated..	end of July	8	32	
	2. Heated ..	but all germi-	17	68	
III.	*Rest of two months*—	nated in the			
	1. Unheated..	rainy week	5	25	
	2. Heated ..	21st–31st July, 1921	15	60	
IV.	*Rest of four months*—	No record kept for number.			
	1. Unheated..		20	80	
	2 Heated ..				

It will be noticed that the seeds heated in the oven germinated better than the others, treatment 3 giving a 72 per cent. germination.

Another experiment, the method and results of which are recorded in Table I, was carried out from April 12th to 30th, 1921. The following interesting points emerge—

(1) The heated seeds germinated better than the unheated.

(2) The long rest apparently helped the germination of the seed.

Simultaneously with this experiment in petri dishes a duplicate set was sown in pots, but these did not germinate until the very rainy period of July 21st to 31st, 1921. The fact of their germination was then noted but no counts were made. These pots were watered daily but only the actual rains stimulated their germination.

In another experiment seeds collected on March 2nd, 1921, were sown at once in pots. One pot was placed out in the sun and the other in the veranda sheltered both from rain and direct sunshine. The pot in the open was not watered, and that in the veranda was watered twice a week. In *neither* pot was there any germination until July 23rd, when germination began in both pots. Graph 1 shows the rainfall, humidity and maximum shade temperature at the time of these two last mentioned experiments.

In December, 1921, the seeds from 100 inflorescences were sown in pots, one pot being devoted to the seeds of each inflorescence. These pots were not watered and were kept in the open. Germination began on July 4th, 1922. Graph 2 shows the rainfall, humidity and shade temperature at the time. During this period there was also copious germination of lavala seeds in the fields. The total number of seeds sown in this experiment was 22,086, but the average germination was only 1·5 per cent. The greatest percentage of germination from any single inflorescence was 8 per cent. Many of these seedlings withered.

In November, 1922, there were unusual rains (Graph 3). During this period further germination took place in the above pots containing the seed of the 100 inflorescences. No exact counts were made, the amount of germination being small. Observation of germination in the field during the monsoon of 1923 generally corroborated the 1921–1922 observations.

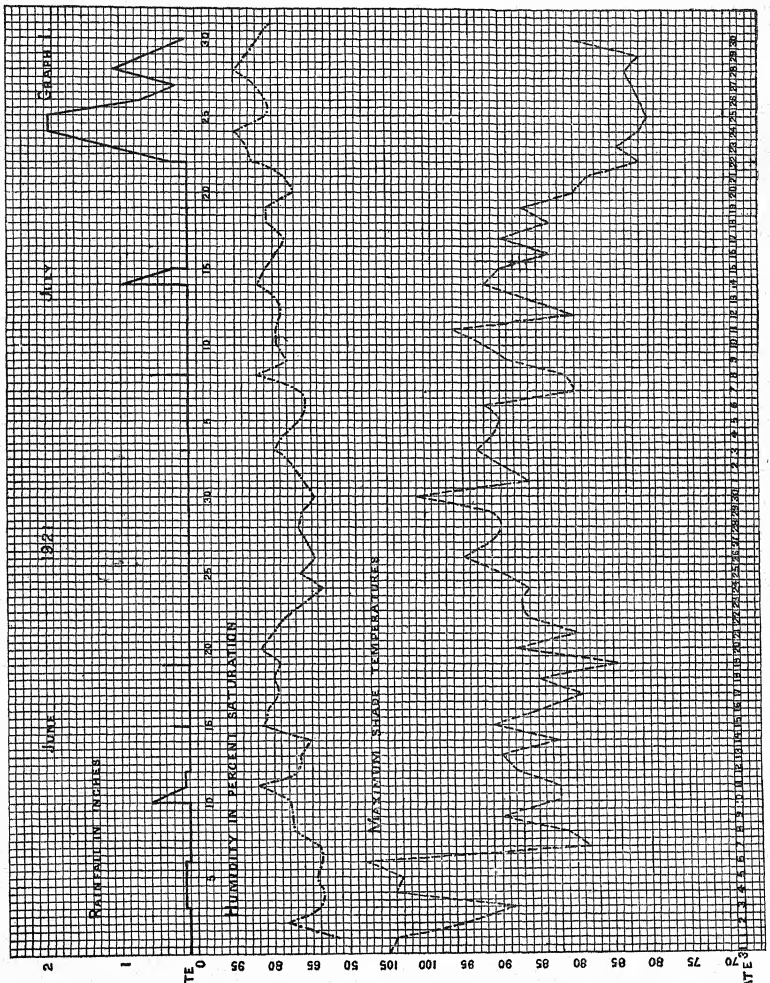
The above experiments lead us to the following conclusions :—

(1) Soil moisture without atmospheric humidity will not cause germination.

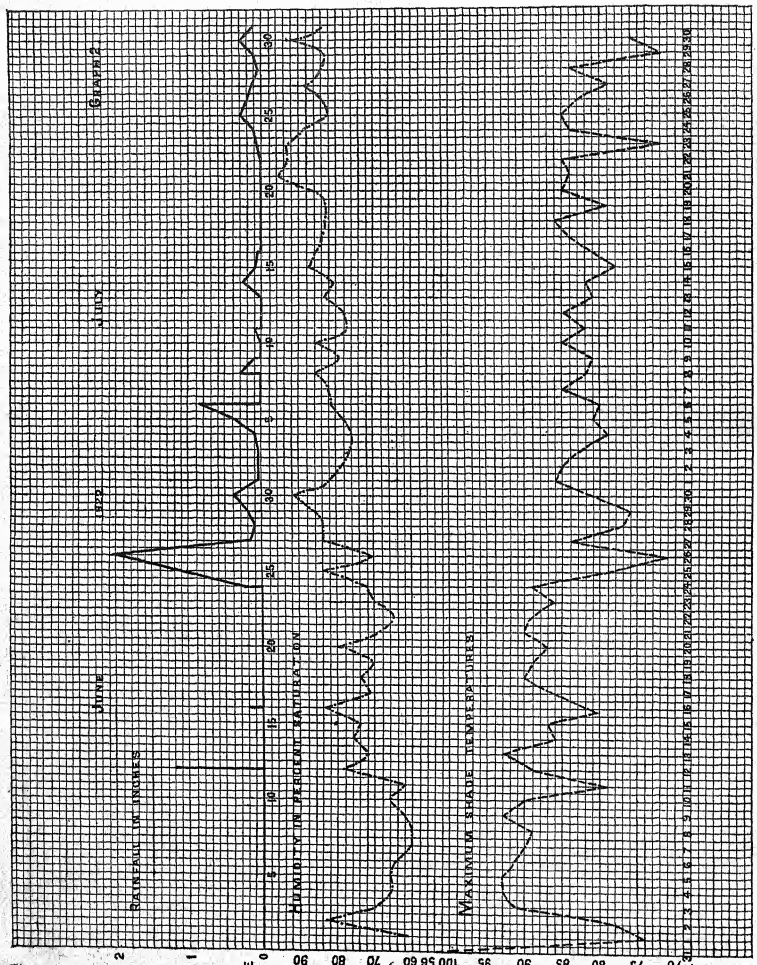
(2) In the field the rainy season is therefore the only one when much germination of seeds will occur.

(3) Even in the rains the actual germination percentage is low, and many seedlings die.

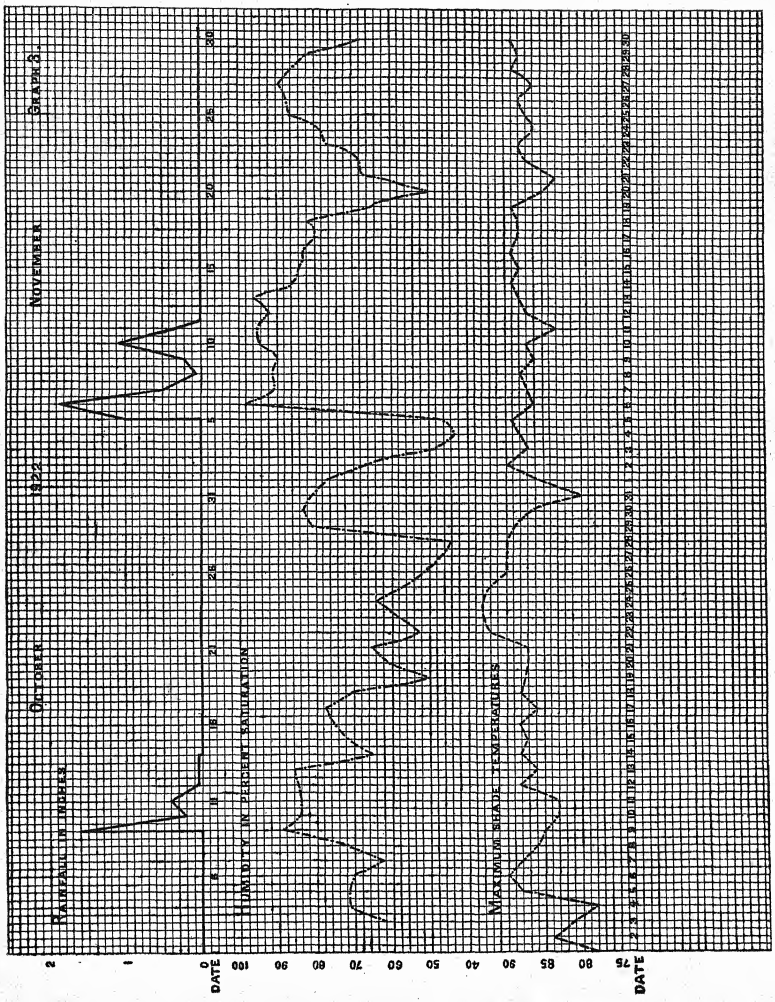
(4) Propagation by seeds is not therefore the main method of propagation, but on account of the enormous total amount of seeds produced per acre, this method of propagation cannot be overlooked and must be checked by methods designed to eradicate this weed before it sets seed.


GRAPH 1.
JULY
1921
JUNE
RAINFALL IN INCHES
HUMIDITY IN PERCENT SATURATION
MAXIMUM SHADE TEMPERATURES
DATE
DATE

GRAPH 1.


GRAPH 2
JUNE
JULY
RAINFALL IN INCHES
HUMIDITY IN PERCENT SATURATION
MAXIMUM SHADE TEMPERATURES
DATE

GRAPH 2.


GRAPH 3.
1922
OCTOBER
NOVEMBER
RAINFALL IN INCHES
HUMIDITY IN PERCENT SATURATION
MAXIMUM SHADE TEMPERATURES
DATE
DATE

GRAPH 3.

IV. Experiments in the propagation of *Cyperus rotundus* by tubers.

Recognising that tuber-propagation is the main method by which this weed spreads itself, we made a large number of experiments of various kinds on the germination of the tubers and the after-growth of the shoots developed from germinated tubers. The general behaviour of the tuber in germination and of the shoots in after-growth have already been briefly described. Our experiments were designed to get the maximum amount of information regarding the life-cycle.

The experiments may be briefly classified as—

(1) Experiments in the germination of isolated tubers,
  (*a*) of different ages,
  (*b*) dormant or sprouting,
  (*c*) at different depths in the soil.

(2) Comparison of the germination of isolated and connected tubers.

Experiment I.

On June 10th, 1920, five black dormant tubers and five white (fresh) dormant tubers were placed in a shallow dish with a little water. This dish was not covered and was kept in the laboratory. In five days all the tubers had slightly increased in size due to water absorption. The buds also were slightly swollen. During the next five days buds elongated to form green shoots. In all the fresh tubers and in all the black tubers in which the apical bud had not previously germinated, the apical bud was the first to sprout. Thereafter the other buds sprouted in acropetal succession. The buds are conical and covered with pointed scale leaves. After the true leaves began to form adventitious roots arose from the base of the buds.

Experiment II.

On July 15th, 1920, ten dormant tubers were placed on top of moist sand exposed to light and ten were planted, one to three inches deep in moist sand. Those on top behaved like the ones described in Experiment I, except that root production was more copious. All buds including those on the under side of the tubers produced shoots which were markedly negatively geotropic.

Shoots from the tubers buried one inch deep appeared on the surface on July 20th. The more deeply buried ones showed shoots above the surface on July 25th. In all but two tubers only one bud had sprouted, this bud being

either the apical bud or the one next to it. In two tubers two buds sprouted simultaneously. Root formation from the tuber nodes was copious. The formation of the vertically growing rhizome between the tuber and the tuft of leaves, and the development of the swelling (basal bulb) at the base of the tuft were first observed in this experiment.

On July 25th, all ten buried tubers were dug out for examination, and five of these were replanted in sand and five in black soil on the same date. At this stage, in addition to the vertically growing rhizome ending in the tuft of leaves, some tubers had developed other rhizomes one or two inches long and certain of these were positively geotropic. At the time of replanting, these tubers were deliberately put in upside down. On August 10th, the plants were again dug out and re-examined. Some of the reversed rhizomes had once more turned down and some had not. Those which had turned down had each formed at their ends a white tuber covered with fibrous scales. The others had formed shoots on the surface. The plants in the black soil had leaves larger and of deeper green than those in the sand. All plants were replaced in the sand or soil and left undisturbed until December 10th, 1920, when they were finally dug out. During their life-time they had been watered twice a week. Flowering took place in the second week of August. It is possible that the transplanting may have accelerated the flowering. All shoots in the sandy soil flowered, but only two in the black soil. In both soils, although watered, the aerial shoots died in September and October. In the second week of November the dormant tubers (both new and old), as also the aerial parts, began to sprout.

When the plants were finally dug out the five tubers in the sand had increased to twenty and the five in the black soil to fifty. Those in the sand were more closely set than those in the black soil.

Experiment III.

In a piece of unirrigated land on the College Farm, 300 dormant isolated tubers were planted in three lots of 100 each at three inches deep, with intervals of four inches each way, on August 10th, 1920. The tubers were not artificially watered but received 10·38 inches of rain during the experiment. Shoots appeared above ground from September 3rd, 1920. Some of these flowered and all began to wither by the beginning of December. The plants were removed carefully and studied for the sequence of tuber formation. Much of the morphological information recorded on page 109 was obtained from this planting.

Experiment IV.

On September 13th, 1920, a set of 100 dormant isolated tubers was planted at 6 inches deep and 4 inches interval in a plot similar to that of Experiment III. These received rainfall of 9·94 inches. Aerial shoots appeared from October 15th. The greater time for the appearance of these is probably caused by the greater depth of planting. The plants were dug out on March 21st, 1921. All aerial parts had by that time withered. Some tubers had remained dormant. Those which germinated had all first produced the upward-growing rhizome ending in the leaves and thereafter tuber-forming rhizomes both from other buds of the original tuber and from the basal bulb. From both these places roots were also developed. One or two of the original tubers had become exposed and in their case all the buds sprouted to form shoots, without any downward-going rhizomes.

These preliminary experiments give the following indications :—

(1) All buds on a tuber have the power of germination. In exposed tubers all these may germinate and produce shoots. In buried tubers the apical one if present, if not, the next, and in some cases both, may germinate to produce the vertically growing rhizome ending in the tuft of leaves. In some cases the tuber may not germinate but remain dormant.

(2) The time between the planting of the tuber and the appearance of the shoot above the soil is determined by the depth of planting of the tuber.

(3) The time between the planting of a tuber and flowering may be in the case of the transplanted plants as short as one month. In the field up to two and a half months may elapse between planting a tuber and flowering.

(4) The rate of increase of tubers is considerable even in the unfavourable conditions of the plants which were dug out to see how they were getting on. Plants in good soil develop longer rhizomes and more tubers than those in poor soil.

Experiment V.

Twelve big robust dormant tubers were planted 3 inches deep in pots in ordinary soil on June 15th, 1921, and finally dug out on August 29th, 1921. Between these dates they were dug out once a week to observe development and Table II shows the results.

TABLE II.

Experiment V.

To study tuber development.

Tubers planted on June 15th, 1921. Plants dug out on August 29th, 1921.

Label No.	Germination of original tuber	Inception of the first new tuber	Sprouting of the first new tuber	Formation of second new tuber	REMARKS
65	20-6-21	1-8-21	13-8-21	5-8-21	
66	,,	29-6-21	1-8-21	1-8-21	
67	,,	24-6-21	29-6-21	29-6-21 to 1-8-21	
68	,,	29-6-21	20-7-21	5-8-21	
69	,,	20-7-21 (tip broken, so no tuber) 1-8-21	5-8-21	13-8-21	
70	27-6-21	20-7-21	1-8-21	,,	
71	20-6-21	5-7-21	20-8-21	20-8-21	
72	,,	20-6-21 (dead) 1-8-21	13-8-21	13-8-21	Flowered 20-7-21
73	,,	20-7-21 From basal bulb 1-8-21 From tuber	No shoot	,,	
74	,,	29-6-21	1-8-21 (Sprouted with a tuberous swelling near the side of the pot)	5-8-21 (2 tubers, one sprouted)	Flowered 5-8-21.
75	24-6-21	,,	25-7-21 (2 white shoots one with a sprouting tuberous swelling)	10-8-21 (the disconnected tuber produced one downward branch)	
76	,,	1-8-21 (dried)		12-8-21 (New tuber from parent tuber)	

The following facts appear:—

(1) Out of the twelve tubers nine germinated within five days of planting and the average time was 6·25 days.

(2) Two plants flowered 35 and 51 days respectively after flowering.

(3) The average time for the inception of the first new tuber was 22·4 days.

(4) The average time after the inception of this tuber for it to sprout was 23·5 days.

It will be particularly observed that there was little pause between the formation of a tuber and its further germination. This fact was confirmed by further pot experiments in which isolated white newly formed tubers were planted and germinated immediately, giving plants more vigorous in growth than those from old black tubers.

Experiment VI.

A. An experiment in planting chains of tubers in pots.

Twelve pots were filled with earth and a small chain of three connected tubers planted in each. Four chains were planted horizontally, four vertically with the youngest tuber upward, and four diagonally across the pot of which two had the oldest tuber uppermost and two had the youngest tuber uppermost.

In the horizontally placed chains the oldest tuber sprouted first. In the vertically placed chains the upper one sprouted first, the lowest remaining dormant or sprouting late. In those placed diagonally the two end tubers sprouted before the middle one. The position in the chain and the depth of planting seem to have therefore some effect on the germination.

B. A similar experiment was started in the field with longer chains of tubers, planted so that the tubers were from 3 to 9 inches below the surface, and any existing aerial shoots in their normal position. These aerial shoots withered, and new shoots replaced them. Planting was done from September 25th to October 2nd, 1920. The rainfall during the experiment was 8·30 inches. The new shoots finally withered in February 1921. An attempt was made to keep a record of the appearance of these shoots, but the record soon became unmanageable. On digging out the plants, however, some useful information was obtained, of which the following notes are given.

(1) Replacement of the aerial parts after withering.

The withering of the leaves may or may not mean the death of the terminal growing point. If this remains alive then when conditions are favourable it

may continue the formation of leaves as before. In addition, since the actual attachment of the aerial leaves to their stem is somewhat below the soil, there may arise new shoots from the primordia in the axils of these leaves and such may develop before the terminal growing point renews its growth, or in the event of its death. Each such new shoot develops its own basal bulb.

New shoots may be formed by the production of a rhizome from the basal bulb or from the vertically growing rhizome below the basal bulb whose leaves have died. These rhizomes make for the surface and there develop new leaves. The point of origin of such a new rhizome from the vertical rhizome of the original plant is sometimes marked by a swelling like an incipient tuber.

(2) Water supply of the aerial parts.

Adventitious roots are developed, as we have seen, from the basal bulb and also from the rhizome and tuber nodes. One case, however, was found in this experiment when there were no roots from the basal bulb and the aerial parts were apparently getting all their moisture from the roots of the tuber one foot below the surface. This is probably a normal occurrence when the surface soil dries out.

(3) Effect of cracks in the soil.

The vertically growing rhizome normally drives its way to the surface and there produces leaves. In very cracked soil if a vertical rhizome pierces a crack the stimulus of the light causes the leaves there to develop, but somewhat faultily, so that they emerge from the soil in a curiously twisted manner.

(4) Two curiosities.

(*a*) One of these was a plant which had sent to the surface the normal vertical rhizome, and this had there formed shallowly placed tubers close together in quick succession, all of them showing traces of aerial leaves.

(*b*) The other was a tuber which bore ten old leaves when planted. This tuber produced one more tuber which in turn developed one aerial shoot and *six* droppers.

1. *Experiments in Deep Planting of Tubers.*

From indications got in this and previous experiments it was decided to make a thorough test of the effect of depth of planting on tubers. These experiments are now recorded.

Experiment VII.

An experiment in the planting of tubers at various depths.

Three rows were arranged each containing eight plots, each three feet square, and in each square plot were planted ten old dormant isolated tubers.

Those in the first row were planted 2 ft., those in the second row 2½ ft. and those in the third row 3 ft. below the surface. The rainfall during the period of the experiment was 8·30 inches. Table III shows the main results of the experiment as revealed when the plants arising from these tubers were dug out on the dates mentioned.

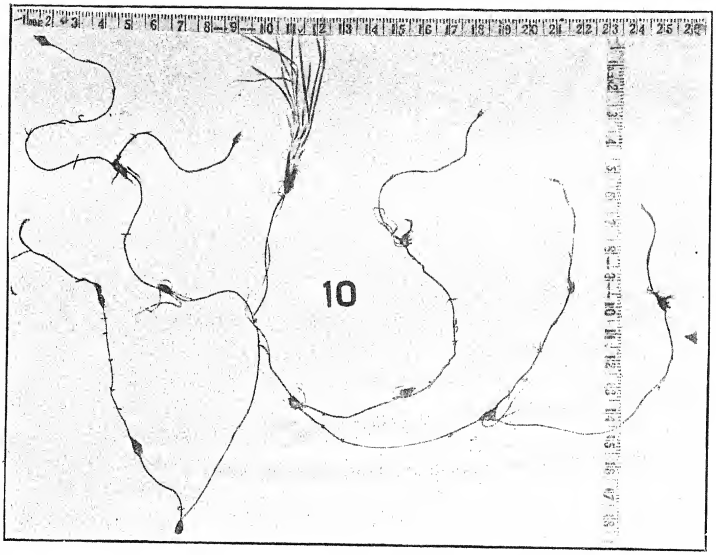
The most salient fact in this experiment is that none of the tubers at 3 ft. deep were able to send their shoots to the surface. Of the 80 tubers in this series only 2 were living at the end of the experiment, 32 were recognisable but empty and shrivelled, and the others (of which shreds were found) had so disintegrated as to be unrecognisable.

The next noticeable point is the marked disadvantage of the plants at 2½ ft. as compared with those at 2 ft. This is clearly shown in the smaller number of tubers in the upper three inches, still more in the smaller number in the middle layers, and also in the smaller number of aerial shoots.

TABLE III.

Planting to the depths 24 *and* 30 *inches on October* 5*th*, 1920. *Percentage of germination and density at various depths.* (10 *tubers in each square*).

Row No.	Sq. No.	Depth of planting (20 tubers)	Date of planting	Dug out on	Original tubers		Shoots on the surface	Total number of tubers in the first 3 inches
					Living	Sprouting		
I.	1	2 feet	5–10–20	5–7–21	1	1	10	6
	2	,,	,,	,,	4	4	10	15
	3	,,	7–10–20	7–7–21	2	2	5	7
	4	,,	,,	8–7–21	1	1	6	8
	5	,,	,,	9–7–21	4	4	5	10
	6	,,	,,	10–7–21	5	5	5	5
	7	,,	,,	,,	5	5	5	10
	8	,,	,,	11–7–21	4	4	10	13



Two rhizomes take off directly from the middle vertically growing rhizome.

TABLE III—*contd.*

Row No.	Sq. No.	Depth of planting (20 tubers)	Date of planting	Dug out on	Original tubers		Shoots on the surface	Total number of tubers in the first 3 inches
					Living	Sprouting		
II.	1	2½ feet	7–10–20	12–5–21	4	2	2	3
	2	,,	,,	15–5–21	5	2	3	2 near the surface
	3	,,	,,	1–6–21	2	2	2	3 (2 basal swellings and 1 tuber)
	4	,,	,,	,,	2	2	2	3 (1 new tuber which soon died)
	5	,,	,,	5–6–21	4	4	4	4 (Basal swellings)
	6	,,	,,	7–5–21	6	2	4	2 basal swellings and 3 new tubers
	7	,,	,,	13–4–21	8	8	4	4 (Basal swellings)
	8	,,	,,	3–5–21	4	10	4	4 (Basal swellings)
					Recognisable Tubers.	Sprouting		
III.	1	3 feet	9–10–20	14–5–21	8	1	..	No tubers in first three inches in any case and no shoots on surface.
	2	,,	,,	,,	6		..	
	3	,,	,,	16–5–21	1	1	..	
	4	,,	,,	20–5–21	1		..	
	5	,,	,,	,,	3		..	
	6	,,	,,	13–5–21	9		..	
	7	,,	,,	12–5–21	7	1	..	
	8	,,	,,	10–5–21	5		..	

The following general observations arise from a study of the 2 ft. and 2½ ft. plantings.

(*a*) The same three points of origin of tubers, as previously observed, were again in evidence, viz.

(*i*) tubers might arise from rhizomes originating from the basal bulb.

(*ii*) tubers might arise from rhizomes originating directly from the vertically growing rhizome. (Plate IV.) In most cases such secondary rhizomes took off in the top three inches of soil.

In both cases these rhizomes might grow definitely downward from the start, when they would be classed as droppers, but the rhizomes taking off directly from the vertically growing rhizome often grew horizontally. (Plate V.)

(*b*) Once the originally planted tuber had established its aerial connection it did not produce further shoots and left the production of new aerial parts to the younger portions of the colony.

(*c*) In one case a tuber planted at 2½ ft. showed swellings, apparently incipient tubers, on the vertical rhizome.

(*d*) Tubers, which either did not germinate or which had germinated and whose shoots had not reached the air, were either shrivelled and devoid of starch or dead although plump. The viability of these later was tested by re-sowing them in pots at a shallow depth and watering them. They did not germinate. When sown they possessed starch but two months later none could be recognised. Later experiments indicate that if the original buds of the tuber are destroyed, adventitious buds are not formed and the tuber, though plump, does not germinate.

It is obvious that such important indications as those given by the series of experiments just described had to be confirmed, and hence further experiments were laid out. These are now described in detail.

Experiment VIII.

On December 4th, 1920, a pit 3×3 ft. was dug out to determine the number of tubers per cubic unit of soil. At the time of refilling of the pit 28 old dormant tubers were placed at the bottom of the pit and earth, free of tubers, filled in on top. The pit was again dug out on April 13th, 1921. No shoots had up to then appeared on the surface. There had been slight rains on January 15th and 16th and thunderstorms on April 3rd and 12th. The soil up to three inches deep was wet, then the layer up to 2½ ft. was dry and the last layer wherein lay the tubers was wet with a capillary moisture content of ten per cent. (calculated on the wet weight). Most of the tubers had produced upward growing rhizomes in an attempt to reach the surface. In seven cases these rhizomes showed tuberous swellings with developing buds, and in two cases typical tubers (not mere swellings) were produced. After an hour the tubers and their growths were returned to the pit and the earth filled in again. The pit was dug out for the second time on August 13th, 1921, after a total rainfall of 11·52 inches since the first excavation. No shoot had appeared on the surface, and all tubers, both the original ones and those formed later, had died.

The following observations were made:—

(*a*) Again the tubers planted three feet deep did not succeed in reaching the surface. In the present case their attempt was interrupted by the first digging.

(*b*) Again the terminal bud of the tuber sprouted to form the upward-growing rhizome. Other buds had sprouted later, possibly stimulated by the check to the first formed rhizome. No descending rhizomes were formed.

(*c*) From this and other experiments we think we are safe in stating that a tuber does not produce a descending rhizome until it has established its aerial connections.

(*d*) Where the growth of the apical bud of the vertical rhizome had been hindered by some hard object there was a tendency towards tuber formation at the part in contact.

(*e*) In growing through hard dry soil the vertical rhizome had a twisted appearance.

(*f*) The younger parts of the vertical rhizome were anatomically complete, but the older parts were bounded by the endodermis, all parts external to it having decayed.

Experiment IX.

This was conducted as follows:—

The soil of the plot was deep black and well aerated, having been twice dug out in previous experiments. Eight rows of eight squares were arranged, each square being 3 × 3 ft. The depths of planting in the successive rows were 3, 6, 9, 12, 18, 24, 30 and 36 inches. Only four tubers were planted per square in order that they might make their maximum growth. Each tuber was planted in a separate hole half an inch in diameter and the proper depth. This depth was checked by lowering the tuber at the end of a string of the proper length. Even with this precaution, at the end of the experiment, tubers were nearer the surface in some cases than at the start of the experiment. This was due partly to washing of the soil and partly to the packing of the soil by the repeated trampling which it received.

The tubers were planted on July 20th, 1921. The first shoot appeared on August 15th, 1921, and the plants were dug out in April 1922. Each plant was carefully excavated, measurements and plottings being made as the work advanced, and the plants mounted as nearly as possible in their natural position and photographed afterwards. This experiment was one of the most illuminating as to the method of growth of the plant from tubers.

The following are some observations.

(*a*) Germination.

Table IV shows how and when the shoots appeared. From this table it is clear that the depth 9 to 18 inches was a layer favourable for vigorous growth of the parent tubers and the plants derived from them. By the end of August all tubers in the depths 3 to 12 inches had sprouted. The reduction

in the germination percentage at the lower depths in this experiment and in Experiment X is noticeable. The tubers in both experiments were brought from the same area in the Empress Gardens, Poona.

TABLE IV.

Planting in holes at different depths on the Poona Agricultural College Farm.

Depth of planting in inches	Tubers planted	No. of shoots	Tubers germinated	First appearance of shoots	Date of final observation
3	32	38	28 Growth poor and less	15th August	22- 8-21
6	32	34	29 extensive	19th ,,	,,
9	32	29	30 Growth very	29th August	20-10-21
12	32	16	15 vigorous and	30th ,,	,,
18	32	9	9 extensive	1st week of September	
24	32	16	16 Growth poor	Middle of September	20-10-21
30	32	4	4 and less	1st week of October	,,
36	32	2	2 extensive	,,	,,

Experiment X.

This was to determine the germination and also the density of production of new tubers at different levels. Twenty-four squares of one square metre were plotted in four rows of six squares each. The following were the depths at which planting was done, three squares being devoted to each depth: 3, 6, 9, 12, 18, 24, 30 and 36 inches. Murum occurred at 18 inches and, in depths below this, the pit was, on refilling, filled with black soil. 400 tubers were planted regularly in each square at the depths mentioned. This experiment was carried out in Modi Bag, the fruit area of the College Farm. The tubers were dug out of the highly infested area of the Empress Gardens, Poona, covered with moist earth. The tubers were washed, dried with cloth and weighed. On placing in the pits a little water was sprinkled on them and the earth then filled in. The earth was moderately packed but not rammed. The experiment started on 6th June, 1921, and the pits were dug out finally from 13th to 21st October, 1921, when most of the aerial shoots were withering. The rainfall before planting was 2·96 inches and during the experiment was 14·45 inches.

Shoots began to appear first in the first week of August in the squares with 12 inches deep planting. This was followed by sprouting in the 18 and 24 inches squares, the latter in the last week of September. The squares with planting at 30 inches produced no aerial shoots till November. The 36 inches deep squares showed shoots on 28th and 29th of September, 1921.

Shoots from the 3, 6 and 9 inches squares began to appear from the second week of September. The shoots were few and with a small number of leaves. The leaves were narrow and not vigorous. These squares and also the 30 inches squares (which had not then shown shoots) were left undisturbed until July 1922, when they were dug out, having received 5·26 inches more rain.

These results call for the following remarks :—

(1) Poor germination at 3, 6 and 9 inches.

This is directly contrary to the majority of our experiments with planting at these depths. The only likely explanation is to be got from a perusal of the rainfall and temperature graphs for the month of June (Graph 1). It will be observed that soil and air temperatures were high at the time of planting and again at the end of the month, and that with the exception of the half-inch rainfall on the 10th and 20th there was little precipitation. The soil, moreover, was only moderately packed and not rammed and hence probably more conductive of heat. It is, therefore, possible that the dryness and heat of the period caused the lack of germination. This, however, is only suggested as the one explanation that at present appeals to us. All these squares showed droppers which seldom penetrated below 18 inches.

The 12 inches deep squares showed the best germination but even here the percentage of germination was low.

In the 12, 18 and 24 inches squares the shoots after reaching the surface had produced tubers and chains of tubers in the 9 inches to 18 inches levels.

Table V shows that the depth 9 to 18 inches was a layer favourable for the vigorous multiplication of tubers, and for the plants derived from them. By the first week of October all the tubers in the depths 3 to 12 inches had sprouted. The very great difference in germination percentage between the tubers at these depths in this experiment and in the one immediately previous is noticeable. The tubers in both experiments were brought from the same area in the Empress Gardens, Poona. Both lots, however, were for all practical purposes alike in quality and kind. The difference in germination may be due to one or all of the following factors :—

(1) (and probably most important) the much more favourable rainfall and temperature at the time of the inception of the other experiment ;

(2) the much better soil in the other experiment ;

(3) the wider planting.

In the squares devoted to tubers planted at 30 and 36 inches depths growth was on the whole poor. The shoots produced persisted forming successive leaves while the old leaves died, but there was no multiplication of shoots.

The low germination percentage at these depths again seems to indicate that some tubers are specially adapted to germinate at lower depths and that these are specially formed by the plant on deep "droppers."

TABLE V.

Planting tubers at different depths on July 6th, 1921. Germination of original tubers and density of new tubers at different levels.

Row No.	Quadrat No.	Depth of planting 400 tubers in each quadrat	No. of tubers germinated	Total No. of new tubers with average at each depth	DENSITY AT VARIOUS DEPTHS			
					Surface to 6″.	6″ to 9″	9″ to 18″	18″ to 24″
I.	1	3″	10	52 } 42	10	30	10	2
	2	,,	23	46 }	7	16	20	3
	3	,,	11	29 }	4	10	15	0
	4	6″	13	53 } 52	6	22	24	1
	5	,,	41	78 }	9	29	39	1
	6	,,	35	26 }	4	8	12	2
II.	7	9″	53	146 } 69	69	53	18	6
	8	,,	2	26 }	10	3	13	0
	9	,,	5	35 }	9	9	17	0
	10	12″	130	155 } 87	40	40	70	5
	11	,,	25	55 }	10	10	25	0
	12	,,	43	52 }	26	5	20	1
III.	13	18″	30	72 } 57	39	20	10	3
	14	,,	27	48 }	24	16	4	4
	15	,,	9	50 }	15	10	16	9
	16	24″	4	60 } 87	8	10	22	20
	17	,,	4	63 }	4	8	25	26
	18	,,	29	138 }	21	6	40	71
IV.	19	30″	12	31 } 32	15	2	10	4
	20	,,	18	50 }	20	4	23	3
	21	,,	7	16 }	10	0	6	0
	22	36″	3	8 } 5	4	0	4	0
	23	,,	3	5 }	3	1	1	0
	24	,,	6	2 }	2	0	0	0

Experiment XI.

In all the experiments about the germination of tubers buried to different depths, recorded in the previous pages, the tubers were planted in pits. To locate more certainly the planted tubers both at time of planting and at time

of digging out, two wooden boxes and one iron box were placed in a large pit dug in the Lavala Experiments Plot with wire floors at different depths.

The boxes were 3 ft. × 3 ft. × 3 ft. and wire gauze pieces 3 ft. × 3 ft. were placed at every 6 inches depth on stretched wires. The meshes of the wire gauze allowed the rhizomes to pass through but hindered the shifting of the tubers.

Each box was devoted to two depths.

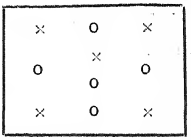
The following table gives the dates of planting and the number of tubers planted at these depths.

Date	27-7-23	I	Wooden box 3′×3′×3′		Five tubers planted at the depth of 6 inches
,,	..	I	,,	do.	1 foot
,,	17-7-23	II	,,	do.	1½ feet
,,	..	II	,,	do.	2 ,,
,,	16-7-23	III	Iron box	do.	2½ ,,
,,	..	III	,,	do.	3 ,,

The ten tubers in each box were laid down in such a way as not to interfere with the growth of each tuber.

× Tubers at the lower level.

O Tubers at the higher level.



The tubers were planted at their proper depths and the soil was put in and uniformly packed. The gauze was placed before fitting the upper layers. The boxes were watered in the beginning. The boxes were finally dug out from 20th November to 30th November. The soil was carefully removed and the original tubers with their subsequent growth were kept intact showing visibly the growth of the plants and the formation of tubers to various depths.

Box 1	Original No. of tubers	Depth of planting	Germinated	Empty	Details
	5	6″	3	2	Tuber (i) Vertical rhizome with no new growth. (ii) Empty. (iii) Vertical rhizome with a sprouting tuber in the axil of the swollen shoot. (iv) Vertical rhizome and a chain of two tubers horizontal to the depth of 6 inches. (v) Not found.

Box I	Original No. of tubers	Depth of planting	Germinated	Empty	Details
	5	1″	3	2	Tuber (i) Empty. (ii) Two vertical shoots one with a swelling at 6″ depth with a horizontal rhizome with a tuber and one ascending rhizome with a tuber. (iii) One vertical shoot with a dropper of one tuber to the depth of 1½ ft. (iv) Vertical rhizome with a new shoot at the surface. (v) Empty.

Box II	Tubers planted	Germinated	Details
			Tuber
18″	5	5	(i) One vertical rhizome swelling at 9 inches producing one more shoot. Horizontal chain at 18 inches with 2 tubers, the middle one producing a vertical rhizome with a tuber at 16 inches. (ii) One vertical rhizome with a swelling at 12 inches. One horizontal chain at 18 inches with one tuber which produced four tubers in the layer 18″–24″, one of which again sent a rhizome reaching the surface. (iii) A vertical rhizome and a dropper with a tuber at 24 inches. (iv) A vertical rhizome. (v) A vertical rhizome with a swelling at 12 inches producing a horizontal chain of 12 inches.
24′	5	4	(i) One vertical rhizome. Another vertical rhizome with two tubers at 20–18 inches. (ii) A horizontal chain of two tubers, the middle one producing a vertical shoot. (iii) Empty. (iv) One vertical shoot with two tubers near the surface, the middle one sprouting. (v) Remarkable growth—straight vertical rhizome with a swelling at 9 inches. A dropper of 3 tubers from the original, the middle of the three producing two tubers on a horizontal chain at 30″. The first of this growth sending a vertical rhizome, straight to the surface forming a shoot.

Box III	Total planted	Germinated	Empty	Details
				Tuber
30″	5	2	3	(i) Empty. (ii) Vertical rhizome. New shoot at the surface. (iii) Empty. (iv) Empty. (v) Vertical rhizome with a swelling near surface.
36″	5	1	2 empty 2 in shrivelled bits.	(i) Vertical rhizome with a tuberous swelling at 6 inches with roots and one shoot.

The following table gives the number of shoots and tubers formed from each box :—

Depth	Tubers		Shoots
	Original	New	
6″	5	3	3
1′	5	3	4
1½′	5	8	8
2′	5	11	7
2½′	5	1	2
3′	5	1	1
	30	27	25

It was not easy to take photographs of the growth in the three dimensions and the boxes were placed in the museum.

This experiment gave visible evidence and corroborated the results of the first experiments about the germination of the tubers and their subsequent growth and the optimum depth of tuber formation.

2. *Experiments in damaging the tubers.*

The next series of experiments deal with the effect of various operations on the tuber. These were cutting, scraping and exposure to sun heat and oven heat. The ulterior aim of these experiments was to get information as to the

probable behaviour of tubers injured or exposed by implements in the course of field cultivation.

Experiment XII. By Wounding.

Two white tubers were cut into four pieces and planted one inch deep in pots on February 7, 1921. All pieces germinated on February 21.

Two white tubers were scraped so that all tissue outside the cortex was removed. These were planted on February 7 and germinated on March 8.

Two black tubers were scraped and quartered, and planted on February 7. These germinated on March 2.

Two black tubers were scraped and planted on February 7. These germinated on February 16.

All the above were watered twice a week. These results were confirmed by a similar experiment in August 1922.

Experiment XIII.

Tubers were also damaged artificially by pricking and cutting (without actually severing the parts) and then planted. Such wounds are often blocked by gum produced by the tuber. Wounded tubers kept in the sun or in an oven at 98°F. (37°C) for seven days did not germinate. This was probably the effect of the temperature, as we shall show later. Of tubers merely wounded and not exposed to high temperatures the majority germinated.

In tissue near a wound the cells are full of a yellowish fluid and the cells next to these have starch grains degenerating in size and number. The fluid in these cells gives no reaction with hæmatoxylin, eosin, acid fuchsin, or safranin. Methyl green stains it slightly. The fluid may possibly be degenerated starch.

We may conclude therefore that damage by implements even if considerable (to the extent of peeling the tubers and of quartering them) is not likely to interfere with their germination. It may also be pointed out that such damage is likely to be on the whole rare, because of the small size of the tuber as compared with the blade or share of any implement, and because of the protection afforded by the earth.

Experiment XIV. By Exposure.

Tubers collected in the first week of December 1920, were put out in the sun daily from 10 A.M. to 6 P.M. until February 1, 1921, when ten of them were put to germinate in black soil in pots. Another ten were kept in the sun till March 2 and similarly planted. Both pots were watered daily.

None of the tubers germinated.

Experiment XV.

On May 6, 1921, four lots of 20 tubers were used. Each lot was buried three inches deep in dry soil in pots and the pots exposed to the sun on the College veranda.

The result is shown in Table VI. Again none of the tubers germinated. Table VII gives the details of another series.

TABLE VI.

Effect of exposing lavala tubers to sunlight.

Lot No.	Exposed		Exposed for days	Remarks
	From	To		
I	6th May	14th May, 1921	8	All of these failed to germinate though watered every day after planting in good black soil.
II	,,	20th ,,	14	
III	,,	27th ,,	21	
IV	,,	16th June, 1921	40	

TABLE VII.

Effects of exposing lavala tubers to sunlight.

	Lot No. I	Lot No. II	Lot No. III	Lot No. IV
Exposure started ..	30–5–21	30–5–21	30–5–21	30–5–21
Weight of 20 tubers in gram. Before exposure ..	17	15	20	17
Weight of 20 tubers in gram. After exposure ..	5·5	7	7	6
Loss of weight	11·5	8	13	11
Percentage of loss	67·6	53·3	65	64·7
Exposed for days	11	15	24	30
Rains in inches during exposure	0·725	0·915	1·295	1·295
Planted (to test effects of exposure) on ..	11–6–21	16–6–21	24–6–21	30–6–21
Germination	0	0	0	0

Experiment XVI.

A field experiment was now arranged. A piece of ground was laid out in twelve plots of a square metre each (four rows of three plots each). In each square plot 400 tubers were planted. In the first row these tubers were laid on the surface of the soil. In the second row they were buried three inches deep in the dry soil. In the third row the tubers were placed on the surface, but 20 litres of water was sprinkled on them and on the soil at the time of planting. In the fourth the tubers were planted three inches deep and 20 litres of water sprinkled over them at the time of planting and before the addition of the top three inches of dry earth.

The total water contents of the soil layers on which the tubers rested was determined before the sprinkling of the water. The water content in each case was—

Row	I	2·38 per cent.
„	II	7·38 „
„	III	3·62 „
„	IV	6·30 „

It will be seen that the soil was very dry, as one would expect at the end of the hot weather. Such soil should theoretically be a good absorber of moisture and a good conductor of heat. The first rains came on June 3, 1921. By June 15 (20 days after planting) the rain-fall amounted to 0·915 inches. The tubers, therefore, had been exposed before the first rains to eight days continuous sunshine. Shoots appeared in the fourth row but not in the others by June 15 and this condition of things continued throughout the months of June and July in which there were 25 rainy and 36 sunny days. The term rainy day means a day in which there was any rain whatever. Of continuous rain there were only 17 days in the two months. The plots were allowed to remain undisturbed till September 20, 1921, up to which date they had received a rainfall of 13·21 inches. There was still no germination in rows I, II and III. The ground sloped slightly towards plot 12, the last plot of row IV, while plot 11 was well drained. Row IV was dug out on September 20 and the tubers from the other plots collected and weighed. Table VIII shows the results.

TABLE VIII.

Experiment XVI in exposing tubers to sunlight.

Results of Row No. IV.

	Quadrat No.			Total
	10	11	12	
Number of tubers	400	400	400	1,200
Planted on	26–5–21	26–5–21	26–5–21	
Dug out on	21–9–21	22–9–21	22–9–21	
Number of tubers :—				
Dormant — Empty	148	194	116	458
„ Normal	0	9	58	67
Germinated	45	16	155	216
New tubers	249	41	251	541
No. of shoots on the surface	126	45	261	432

Note. Of the original 1,200 tubers in this row only 18 per cent. germinated, 5·5 per cent. failed to germinate, 38·16 per cent. were empty of contents and of the remaining 38·34 per cent. no trace was found. Nevertheless 18 per cent. (216 tubers) which germinated during three months of the favourable season produced additional (541) new tubers. Thus in place of the original 1,200 tubers, 824 tubers (68·6 per cent.) were found at the end of the experiment.

This experiment indicates—

(1) the amazing efficiency of an eight-day exposure to the May sun even at a depth of three inches in dry soil ;

(2) the way in which a moist soil layer at three inches deep keeps the tubers alive ;

(3) the speed with which tubers multiply again.

Experiment XVII.

This experiment was laid out in the same way as the last with the following modifications.

(1) Soil moisture determination was done both before and after the experiment.

(2) In Rows III and IV soil moisture determination was in addition done both before and after the sprinkling of water at the beginning and after the draining off of surplus water at the end of the experiment.

(3) Ten litres of water was used for sprinkling each square instead of 20.

(4) 200 tubers per plot were used instead of 400. Only new tubers at the ends of rhizomes were used. The experiment was started on November 9, 1921.

Soil moisture percentages (total water) were—

Row I	.. ..	19·9
„ II	.. ..	22·5
„ III	before watering	20·2
	after „	37·5
„ IV	before „	20·5
	after „	44·3

Soil moisture percentages at the end of the experiment on December 28, 1921, were—

Row I	17·6
„ II	18·9
„ III	28·7
„ IV	35·2

These plots received neither rain nor irrigation water during the experiment. Again there was no germination in the first three rows and this is all the more striking when one considers the marked increase in the soil water. Row IV did not show germination till November 21. Germination in Row IV was as follows :—

Square 10	..	20 tubers out of 200
„ 11	..	25 „ „ „ „
„ 12	..	3 „ „ „ „
	TOTAL ..	48 „ „ „ 600
	or 8 per cent.	

Rows I to III were left untouched till January 6, 1922, but there was no germination in them.

This experiment confirmed the effectiveness of exposure.

Experiment XVIII.

In the May experiment the maximum temperatures of the atmosphere, surface of the soil and soil layer three inches deep were 108°, 111° and 117°F. (i.e., 42°, 44° and 47°C. respectively). In the November experiment the same sites had the maxima of 101°, 105° and 99°F. (i.e., 38°, 41° and 37°C. respectively). In order to determine in controlled conditions the degree and duration of temperature necessary to destroy the life of the tubers a preliminary experiment was arranged. The range of temperature was 34° to 49°C., i.e., 91° to 120°F. and the duration ten minutes to seven hours. Heating was done in a steam oven. The tubers thus heated were planted in soil at three inches deep and watered. Most of these germinated. The loss of weight due to this artificial heating was 16·8 per cent. In the field experiments the heating was for several days and the loss in weight up to 50 per cent.

Details of the method are these. Lots of ten tubers were placed in paper bags and kept at a constant temperature in the oven for the time required. After heating they were weighed and after one day in wet soil they were again weighed to find out how much water they had reabsorbed. It was found out that they made up their original weight and in many cases exceeded it. Details are given in the Appendix.

If we consider the first case in which the tuber has not absorbed up to its original weight (ten minutes exposure 98°F. (37°C.)) we find that there was no germination. In the next case of the kind, however (one hour exposure at 109°F., i.e., 43°C.), seven out of the ten tubers germinated. The same is the case for 30 minutes at 115°F. (46°C.). In the case of one hour exposure at 119°F. (48°C.), where the tubers had not absorbed up to their original weight the germination was eight out of ten. In the case of 115°F. (46°C.) there is a loss at all durations except the four hour one where the gain is very small, and a much reduced germination. At 121°F. (49°C.) there is a loss on the original weight in three out of five cases, but the germination is much better. From these figures it is plain that one cannot yet come to definite conclusions as to the relation of desiccation to germination. There is, nevertheless, noticeable a tendency, as the temperatures and durations increase, for the desiccation to advance to such an extent that tubers do not make up their original weight after one day in moist soil.

Experiment XIX.

Armed with the above experience we now planned another experiment in artificial heating. Healthy plump tubers from the Empress Garden were used. These were divided into four lots of ten each, and each lot duplicated. One lot of ten of each pair was exposed in a shallow paper dish and the duplicate lot in a similar dish but just covered with dry earth. The temperature of the oven was 104°F. (40°C.) all the time and the four lots were heated for three, seven, eight and fourteen days respectively. The tubers were weighed before and after the heating, then placed in wet blotting paper for a day to measure the amount of water reabsorbed, planted two inches deep in soil and watered to test germination. Heating began on March 20, 1923, and continued till April 4, 1923. Germination was watched till July 2, 1923. For actual weight, etc., see the Appendix.

The main results were :—The percentage of loss of weight increased with the period of exposure and the losses are greater in the series simply exposed in paper dishes than in those exposed in an earth layer.

The reabsorption of water from blotting paper was inversely proportional to the time of heating. Taking both series (open dishes and earth layer) the average of the amount by which the tubers failed to make up their original weights was 20 per cent.

The germination results show clearly that in spite of these losses, heating up to eight days by no means destroyed the vitality of the tubers, while heating up to fourteen days was completely destructive.

Experiment XX.

Still another experiment of the same type was designed to confirm the effects of long exposure to artificial heat. Four lots of ten tubers, again duplicated and again similarly exposed, were put in the oven at the same temperature, this time for fifteen days. Heating began on April 5 and ended on April 20, germination being studied up till July 2. The average deficit of reabsorption for both series was 35·1 per cent. and only one out of eighty tubers germinated, this being one from a paper dish without earth. For details, see the Appendix.

Experiment XXI.

We now returned to the open field for a last confirmatory experiment. The maximum temperature of the earth in March 1923 being 104°F. (40°C.) the same as the oven, we exposed four lots of 25 tubers in pots, one lot on the surface of dry earth, one lot on the surface of wet earth, one lot two inches deep

in dry earth and one lot two inches deep in wet earth. Weights of tubers were again determined before and after exposure. Exposure without moisture lasted only for five days, for there was rain on March 24 and 26 and the pots got a total of 0·16 rain on these two days. Conditions, therefore, rather favoured the germination of the tubers. Nevertheless the tubers fully exposed on the surface of dry soil had all been killed, and the germination of all except the last pot was unsatisfactory. In the last pot the germination was 92 per cent. indicating the considerable advantage derived from the originally wet seedbed.

Summary.

Summing up all these exposure experiments we see that a temperature of 104°F. (40°C.) in an incubator has to be applied for fourteen days to be effective. The same temperature in the field, where tubers are either exposed to the sun or are not deeper than three inches in dry earth, is effective within eight days. This is another important indication for our guidance in dealing with the plant and suggests the need for some agricultural operation which will bring the maximum amount of tubers to the surface in the hottest part of the year.

3. *Experiments on the effect of spraying with various chemicals.*

Experiment XXII.

Since spraying with copper sulphate and other chemicals has been found effective in killing certain other weeds, experiments were designed to test the effect of such spraying on the lavala weed.

These were first done in two ways, (1) by watering plants grown in pots, (2) by mixing the chemical with the soil and then planting tubers in the soil.

(1) Watering plants in pots.

The chemicals used were common salt, quicklime, magnesium sulphate, ferrous sulphate, copper sulphate. These chemicals were administered in 2, 4, 6, 8 and 10 per cent. solution strengths. There were for each treatment two series of pots, one with single tubers and one with chains of tubers. Two pots with single tubers and two with chains, a total of 20 pots for each chemical.

The pots were filled with good river soil on June 21, 1921, and the tubers and chains of tubers (whole systems with the aerial shoots) were planted on June 22 and 23. The plants were watered with ordinary water till June 27.

On that date two and a half litres of the solutions were poured on to the soil in each pot after stirring the soil. The pots were kept under observation till July 27. During this period they received 7·27 inches rain.

The results were as follows :—

Common salt :—All pots showed caked and cracked surface soil. All shoots withered, but those from plants in the pots treated with 2, 4 and 6 per cent. solutions revived, producing new leaves. Unsprouted tubers (whether solitary or in chains) remained dormant till the third week. Then all the tubers in the 2 and 4 per cent. pots sprouted, but in the 6 to 10 per cent. pots only two or three tubers sprouted. In the 10 per cent. pot the few tubers that sprouted did not survive.

Quicklime (slaked) :—This liquid was added to the soil when cool. There was no evil effect on either shoots or tubers. All the solitary tubers sprouted.

Magnesium sulphate :—The effect was stimulation of growth. The solitary and chain tubers sprouted. The shoots were deep green and flowered early and profusely.

Ferrous sulphate :—The shoots turned yellowish and sickly in the first week and later revived.

Copper sulphate :—The shoots turned yellowish and sickly during the first week. The plants treated with 2 and 4 per cent. solutions revived in the second week. The plants with the 6 to 8 per cent. treatment survived till nearly the end of the experiment and then died. The plants under the 10 per cent. treatment died at the beginning of the experiment. Tuber germination was delayed till the third week, but few of the shoots survived in any strength of treatment.

(2) Pots with chemicals added previously to the soil.

The soil in the pots was wet with rain. The moisture was determined and the amount of chemical added so as to be a percentage of the dry weight of the soil. The actual percentages were close to 2, 4, 6, 8 and 10 of the dry weight of the soil in each pot. The same series were employed (solitary tubers and tubers in chains with the aerial parts intact). Planting was done on July 27. The plants were observed for a month during which 3·71 inches of rain fell. The effects were similar to those in the first experiment but were more severe in the case of common salt and copper sulphate. The lime and the magnesium sulphate had no evil effects. In the ferrous sulphate pot the tubers germinated but withered in the second week. In the copper sulphate pots all the tubers sprouted in the third week but withered in spite of the rain.

(3) On the basis of these indications 3×3 ft. quadrats of lavala infested soil were sprayed as follows :—

One with 2 per cent. common salt, one with 2 per cent. copper sulphate, one with hot water and one kept as a control. The hot water treatment was

supposed to be with boiling water. By the time the water reached the plants however, the temperature had fallen to about 62°C. Hence so far as temperature went the treatment was useless. The treatment, however, was continued to the beginning of May 1922. The other treatments started on November 1921 and continued till October 1922 twice a week, being 100 applications in all.

The area between the quadrats was numbered and dug up and the number of tubers in each section counted. This indicated the tuber population per 27 cubic ft. to be 875, a high figure.

The soil was put back into the trenches, but the tubers were not put back. The idea was to keep the quadrats free from encroachment from outside tubers.

The countings showed the decrease of tubers as one goes deeper into the soil and also that there are many more tubers than shoots.

Method :—Each quadrat was sprayed twice a week with a 2 per cent. solution of the chemical.

Spraying was done from 25th November, 1921. After the 46th spraying the shoots in all the quadrats were counted at the time of each spraying and the result is shown in Graph 4. The greater efficiency of the common salt is clearly shown. It is also observable that the number of shoots in all plots markedly decreases after the rains.

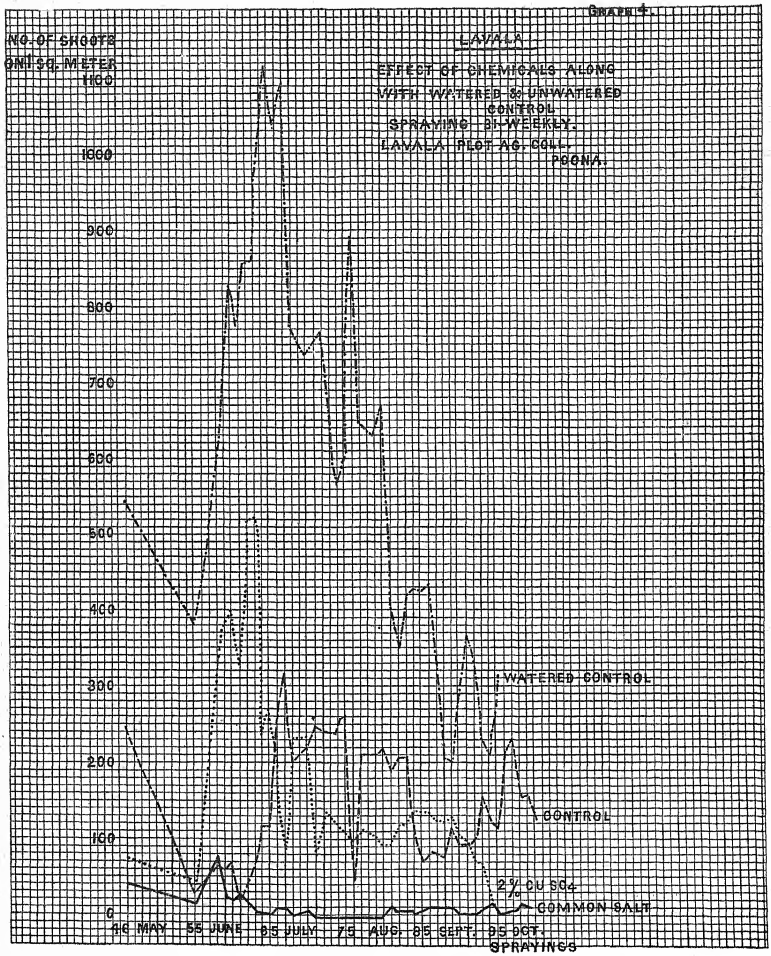
After the 67th spraying the soil at various depths in the salt sprayed quadrat was analysed with the following result.

TABLE IX.

Soil samples.

	I	II	III	IV
Depth	6″	6″ – 12″	12″ – 18″	18″ – 24″
Chemical common salt	**1·77**	**0·53**	**0·046**	**0·046**
		per cent.		

From these same four depths and from this quadrat four pots were filled with soil and in each pot 50 seeds of jowar (*Andropogon Sorghum*) and five lavala tubers were planted to test the growth of these plants in the soil which had been subjected to the spraying. The jowar in all the pots germinated. In pots 1, 3 and 4 the seedlings were sickly, but in pot 3 they improved afterwards and produced plants three feet high which headed out. Pots 1 and 4


GRAPH 4.
NO. OF SHOOTS ON 1 sq. METER
1100
1000
900
800
700
600
500
400
300
200
100
0
LAVALA
EFFECT OF CHEMICALS ALONG WITH WATERED & UNWATERED CONTROL
SPRAYING BI-WEEKLY.
LAVALA PLOT AG. COLL. POONA.
WATERED CONTROL
CONTROL
2% CU SO4
COMMON SALT
1st MAY 15 JUNE 15 JULY 15 AUG. 15 SEPT. 15 OCT.
SPRAYINGS

GRAPH 4.

did not produce earheads. Pot 2 produced healthy plants which headed. These pots were watered daily. There was therefore probably washing out of the salt. Even with this, however, the surface layer seems to have had too much salt for the health of the jowar. The poor growth from pot 4 may possibly be explained by the fact that this was subsoil. In addition, 100 jowar seeds were planted on the remaining undisturbed part of the salt-sprayed quadrat and allowed to get the rain (from 20th July, 1922). The seeds germinated but withered, and only three seedlings on the border where presumably there was less salt grew three feet tall and headed out. The indications are that the surface soil was too salt for the growth of jowar.

The lavala tubers in the pots first mentioned all germinated.

One-fourth of the quadrat was then dug out and the total number of tubers found was 121. The distribution was as follows :—

TABLE X.

Quadrat II (Salt). Area (2·25 sq. ft.)

Depth	0″ – 6″	6″ – 12″	12″ – 18″	18″ – 24″	TOTAL
Tubers	12	38	61	10	121

Five healthy-looking tubers from each depth were selected and planted in good soil in pots on 20th July, 1922. All failed to germinate except three from the lowest layer. The others on 20th August, 1922 were found to be shrivelled. Treating with 2 per cent. salt, therefore, seems to be effective in killing the shoots and the tubers in the upper layers, but it does not kill the tubers which are deeper and it renders the upper layer of soil unfit for jowar.

The application of chemicals was stopped after the 100th spraying (20th October, 1922). During the period from that date to 14th November, 1922, there was an increase in the number of shoots in the salt plot. This was found to be due to invasion from the control plot, the rhizomes having crossed the previously dug out and refilled trench. The remaining three-fourths of the salt plot were dug out on 14th November, 1922, and in the first foot were found 40 shrivelled and 30 plump tubers, in the second 20 shrivelled and ten plump tubers. The plump tubers were all transplanted into good soil the same day in Modi Bag. Only two tubers from the first foot and three from the second foot germinated within a week. The others failed to germinate.

In the copper sulphate plot the chemical seemed to kill the foliage (which was to be expected) as the leaves were made to turn yellow, but these were constantly replaced by new leaves and new shoots. The whole quadrat was dug out on 17th October, 1922 with the following result:—

TABLE XI.

Chemical $CuSo_4$.

Depth	6″ – 6″	6″ – 12″	12″ – 24″
Tubers	248	392	45

4. *Experiments in repeated removal of the shoots.*

The next series of experiments deals with the effect on the tuber of repeated removal of the aerial parts. Such removal of aerial parts is all that occurs in ordinary hand-weeding and in a good deal of weeding by implements, hence it was necessary to determine the effect of such removal. Theoretically, if one could continuously remove all leaves as they appeared one ought to be able to exhaust the reserves of food in the tubers.

Experiment XXIII.

A single tuber freshly dug out was soaked in water for 24 hours and planted two inches deep in black soil in a pot on June 1, 1920. This tuber when dug out weighed 0·87 grm. and after soaking 0·92 grm. The shoot was cut off at intervals by a pair of scissors as close to the soil as possible. The dates of cutting and number of intervening days were as follows:—

Date of cutting	Interval in days
11–6–20	10
3–7–20	23
2–8–20	20
11–10–20 (last shoot)	105
20–12–20 (no shoots)	tuber dug out.

The plant throughout the experiment was watered twice a week.

The tuber when dug out weighed only 0·32 grm. It was shrivelled, only the outer skin and the wood fibres being left. It is remarkable that although the plant was not allowed to develop its shoot the tuber took five and a half months to die. The tuber lay dormant for a long time before making its final attempt to produce a shoot.

Experiment XXIV.

On June 11, 1921, 24 freshly dug tubers were weighed and planted two inches deep in good black soil, each tuber in a separate pot. Shoots were cut off as in the last experiment. Table XII gives the data. No tuber made

more than six successive attempts at shoot production. After three months no shoots appeared. The pots were kept under observation till June 11, 1922, when all the tubers were dug out. Eleven tubers were in shreds and the other thirteen hollow and without starch. There had been no production of new tubers.

TABLE XII.

Effect of frequently cutting off the aerial shoot produced from the Lavala tuber.

Experiment started on 11th June, 1921
ended on 11th June, 1922.

Tuber No.	Label No.	No. of shoots cut at a time	Date of cutting	Interval between two cuttings (Days)	Original weight of tuber	Final weight of tuber
					Grm.	Grm.
1	37	2	20-6-21	9	1·070	0·120
		1	29-6-21	9		
		1	15-7-21	16		
2	38	1	18-6-21	7	1·050	0·257
		1	27-6-21	16		
		1	6-7-21	9		
			22-8-21	47		
3	39	1	27-6-21	16	0·837	0·113
		1	6-7-21	9		
		1	23-7-21	17		
4	40	2	20-6-21	9	1·055	0·807
		1	27-6-21	7		
		1	6-7-21	9		
5	41	1	18-6-21	7	1·104	Shreds.
		1	27-6-21	9		
		2	4-7-21	7		
6	42	1	18-6-21	7	1·052	,,
		1	27-6-21	9		
		2	4-7-21	7		
7	43	2	18-6-21	7	0·905	,,
		1	23-7-21	35		
8	44	2	23-6-21	12	1·050	,,
		1	4-7-21	11		
		1	23-7-21	19		
9	45	1	18-6-21	7	0·950	,,
		2	27-6-21	9		
		1	4-7-21	7		
10	46	1	18-6-21	7	0·870	0·529
		1	23-6-21	5		
		1	29-6-21	6		

TABLE XII—*contd.*

Tuber No.	Label No.	No. of shoots cut at a time	Date of cutting	Interval between two cuttings (Days)	Original weight of tuber	Final weight of tuber
					Grm.	Grm.
11	47	2	20–6–21	9	0·840	Shreds.
		1	27–6–21	7		
		1	19–8–21	53		
12	48	1	18–6–21	7	1·520	„
		2	27–6–21	9		
		2	4–7–21	7		
		1	18–7–21	14		
13	49	1	18–6–21	7	1·200	„
		1	26–6–21	9		
14	50	2	23–6–21	12	1·180	„
		1	4–7–21	11		
		1	18–7–21	14	1·500	
15	51	No shoot was found.		Tubers all in small bits.		
16	52	2	18–6–21	7	1·350	1·020
17	53	2	18–6–21	7	1·285	Shreds.
		1	29–6–21	11		
		1	13–8–21	45		
		1	2–9–21	20		
18	54	2	18–6–21	7	1·287	0·169
19	55	1	16–6–21	5	1·902	0·731
20	56	2	18–6–21	7	1·609	0·329
		1	27–6–21	9		
		1	6–7–21	9		
21	57	1	18–6–21	7	1·409	0·625
		1	27–6–21	9		
		1	15–7–21	18		
		1	28–7–21	13		
22	58	1	27–6–21	16	1·452	0·914
		1	12–7–21	15		
		1	10–8–21	29		
		1	29–8–21	19		
23	59	1	16–6–21	5	1·435	Shreds.
		1	23–6–21	7		
24	60	1	18–6–21	7	1·695	0·642
		1	27–6–21	9		

These two experiments confirm the theory that repeated removal of the aerial parts will kill a tuber. This seems simple. The following experiments will show how the treatment works in actual practice in weeding in the field.

Experiment XXV.

This experiment had a double purpose (1) to test the effect of weeding on lavala colonies in a small plot, (2) to compare the effectiveness of two methods of weeding.

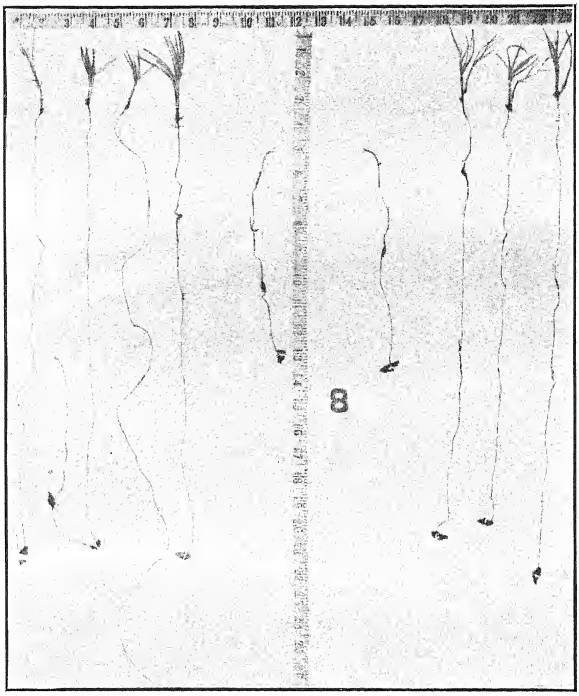
As regards point (2) :—We have shown that each aerial shoot has at its base a bulb (Plate VI, 2) not a tuber, from which arise horizontal rhizomes and droppers. Ordinary weeding by the weeding hook (*Khurpi*) does not remove this bulb. If weeding were done to tear up this bulb then such weeding ought to be much more effective. Accordingly a coarse fork was made which was thrust under the bulb, thus levering it up. One plot was weeded with this instrument and one by the *Khurpi*. (Plate VII, 1.)

The plots were in an unirrigated part of the college farm. One plot measured 2 × 2 metres and the other 2 × 3. They should have been of the same size, but the results are made comparable by reducing them to terms of one square metre. Weeding began on September 7, 1920, and went on till May 16, 1921, when the tubers in each plot were dug out, counted and replaced. No further weeding was done till September 1, 1921; so the transplanted tubers had the full benefit of the 1921 rains. On September 13, 1921, the tubers were finally dug out and counted. The number of shoots visible was counted at each weeding. Table XIII gives the details of the experiment :—

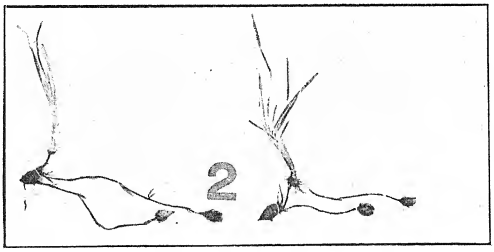
TABLE XIII.

Weeding Experiment.

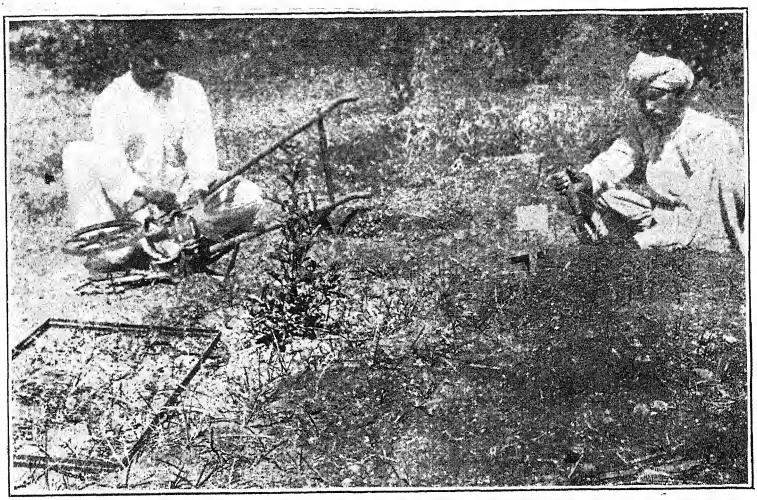
Date of weeding	Days between weeding	No. of shoots removed per weeding by		No. of shoots removed per weeding calculated per sq. metre	
		Area			
		Khurpi 2×2 metre	Fork 2×3 metre	Khurpi	Fork
7-9-20	21	120	205	30	34·1
29-9-20	71	310	355	77·5	59·1
9-12-20	71	190	155	47·5	25·8
8-1-21	30	120	69	30	11·5
7-2-21	30	90	40	22·5	6·6
8-3-21	29	86	26	21·5	4·5
8-4-21	31	56	6	14	1·0
8-5-21	23	73	20	15·7	3·3
16-5-21	Tubers dug out, counted and replaced.				
1-9-21	116	440	270	110	45
13-9-21	12	180	161	45	26·8
13-9-21	Tubers finally dug out.				



1. Plants from deep tubers.



2. The right-hand plant shows the " basal bulb " clearly. One rhizome emanates from it and another rhizome from the tuber below.



1. The man on the right is using the fork.

2. Weeding with Planet Junior Hoe.

Tubers at test diggings.

Date	Khurpi-weeded plot (2× 2 metres)		Fork-weeded plot (2×3 metres)	
	Empty tubers	Normal tubers	Empty tubers	Normal tubers
16-5-21	86	405	172	480
13-9-21	33	113	141	126
	Above figures reduced to one sq. metre as unit.			
16-5-21	21·5	101·25	28·66	80
13-9-21	8·25	28·25	23·5	21

The following points emerge :—

(1) Reduction of shoots. From September 7, 1920, till May 8, 1921, the percentage reduction in shoots by the *Khurpi* method of weeding was only 39·16 ; while by the fork method it was 90·28. This demonstrates the greater efficiency of the uprooting of the basal bulb.

(2) Reduction of tubers. The original number of tubers is not known. The total number of empty and normal tubers found at the end of the experiment cannot be considered as a measure of the number at the beginning of the experiment, since in the *Khurpi*-weeded plot the tubers probably multiplied considerably and in the fork-weeded plot their multiplication was probably much impeded. The figures, however, show some surprises. The first of these is the comparatively small difference in number of normal tubers between the two plots, when compared with the large difference in above-ground shoots. The still more astonishing result is the very small number of sound tubers remaining at the end of the experiment in the *Khurpi*-weeded plot.

One fact stands out prominently. Neither form of weeding had entirely cleared the ground of tubers. There were left enough to repopulate the area in a very short time.

Experiment XXVI.

This was a field experiment to repeat on a larger scale the earlier experiment on the weeding of tubers deliberately planted for the removal of their aerial parts. Pits 3×3×3 ft. were excavated in Empress Gardens and the number of tubers in each layer was counted and set aside. These tubers were

replanted in similar sized pits on the College Farm. In pits I and II, the tubers were replaced at the depths in which they were found. In pit III the tubers were mixed with the soil throughout the whole 3 ft. depth. The surface of pit II was weeded and pits I and III were kept as controls. The experiment started on November 17, 1922, and ended on October 31, 1923, when the tubers were dug out. Weeding was done once a week by the fork. No watering was given. Shoots appeared from January 13, 1923. Table XIV shows the results.

TABLE XIV.

Number of shoots from the tubers transplanted.

Weekly weeding No.	Quadrat weeded weekly II	Control quadrat (tubers in layers) I	Control quadrat (tubers mixed in soil) III
1	67	21	1
2	25	54	4
3	15	4	2
4	10	18	1
5	17	12	2
6	5	19	2
7	6	27	2
8	38	25	8
9	37	24	6
10	80	13	7
11	8	15	6
12	6	9	1
13	3	1	1
14	4	15	3
15	..	..	..
16	2	0	1
17	2	4	5
18	2	0	3
19	0	0	0
20	0	9	2
22	1	2	1
23	0	2	0
24	0	0	1
25	..	..	..
26	11	19	28
27	28	..	..
28	..	..	..
29	118	115	47
30	101	130	50
31	54	121	78
32	8	118	82
33	5	120	86
37	4	124	134
38	1	107	113
39	0	109	121

Judged by the results on the tubers the weeding in pit I has been effective. It must be remembered that these were tubers planted *ad hoc* with aerial

parts removed as soon as shown and were much in the position of the isolated tubers in pots in experiment.

In the two control plots there was a considerable total decrease in tubers and there was a decrease also in the number of viable tubers. This shows that the conditions were much less favourable in their new home than in the Empress Gardens from which they were transplanted.

Experiment XXVII.

Two adjacent plots each measuring 100×20 ft. were selected in a weed-infested area of the College Farm. One of these was used as an untreated control and the other weeded once a week by the Planet Junior Hoe with the ordinary weeding blades on it. (Plate VII, 2.) Before weeding two quadrats each 3 by 3 ft. were selected and the shoots on these counted. These were regarded as fixed quadrats and all countings done on them. Tables XV and XVI give the counts of the shoots in both quadrats and the number of tubers in the soil at the close of the experiment.

TABLE XV.

Number of shoots on the quadrats (3×3 ft.) *in the experiment of weeding with a Planet Junior Hoe once a week.*

No. of weeding	Date	Shoots in quadrat in plot 9 Hoed	Shoots in quadrat in plot 10 Control
1	18–8–22	494	556
2	29–9–22	110	379
3	6–10–22	229	314
4	21–10–22	122	235
5	27–10–22	185	204
6	3–11–22	158	205
7	....	...	..
8	17–11–22	205	138
9	24–11–22	323	254
10	1–12–22	164	253
11	8–12–22	184	396
12	15–12–22	128	105
13	22–12–22	167	393
14	29–12–22	126	243
15	5–1–23	26	341
16	12–1–23	102	105
17	19–1–23	94	327
18	26–1–23	126	364
19	2–2–23	105	297
20	9–2–23	212	245
21	16–2–23	106	107
22	23–2–23	78	312
23	2–3–23	..	102
24	9–3–23	50	310
25	16–3–23	28	217

TABLE XV—*contd.*

No. of weeding	Date	Shoots in quadrat in plot 9 Hoed	Shoots in quadrat in plot 10 Control
26	23-3-23	64	285
27	30-3-23	47	325
28	6-4-23	37	334
29	13-4-23	11	323
30	20-4-23	23	391
31	27-4-23	..	326
32	4-5-23	14	315
33	11-5-23	103	76
34	18-5-23	104	300
35	25-5-23	108	309
36	8-6-23	221	105
37	15-6-23	12	103
38	22-6-23	12	106
	Hoeing not possible due to rains.		
39	3-8-23	210	586
40	10-8-23	510	63
41	17-8-23	635	126
42	24-8-23	370	47
43	31-8-23	362	62
44	7-9-23	489	35
45	14-9-23	453	33
46	21-9-23	483	44
47	28-9-23	282	79
48	5-10-23	397	72
49	13-10-23	356	116
	The experiment was stopped.		

TABLE XVI.

Final test diggings in the experiment of weeding with Planet Junior Hoe, College Farm.

No.	Plot	Date	Shoots on the surface	Surface to 6″		7″–12″		13″–18″		19″–24″		Total of good tubers	Empty
				Normal	Empty	Normal	Empty	Normal	Empty	Normal	Empty		
1 ..	Hoed.	25-10-23	247	220	7	453	5	231	5	122	0	1,126	17
2 ..	„	27-10-23	365	365	26	447	6	221	3	26	2	1,069	37
3 ..	„	28-10-23	295	354	11	435	23	353	12	64	0	1,206	46
4 ..	Control.	25-10-23	308	236	22	318	5	209	2	29	1	792	30
		Grass shoots..	28										
5 ..	„	27-10-23	535	529	6	334	8	336	10	171	3	1,372	27
		Grass shoots..	21										

It will be seen that this weeding was absolutely ineffective. In fact the control plot was less weedy than the weeded plot. This was partly due to the growth of grass on the control plot competing with the lavala. We shall have more to say later on regarding the competition of grass and lavala.

The ground was unirrigated and hard. Hoeing in this superficial manner was doubly unsatisfactory on account of the hardness of the soil and because the shallow-going implement did not uproot the basal bulb. Not only so, but the clearing away of the loose soil after a weeding revealed shoots on their way up which had escaped the hoe blades. An actual count made on January 25, 1923 gave the following figures in a 3×3 ft. square surface.

Number of shoots before weeding	28
,, ,, ,, after ,, ...	7
Two inches of loose soil were then removed and there appeared shoots wholly or partially protected from the hoes	53
(These included 21 of which the leaves had been severed but the basal bulb untouched.)	
In another case the number of shoots before weeding was	35
After removal of two inches of soil there appeared (including the above 35)	129

As it is manifestly not economic to scrape the loose soil off and then hoe, this method of weeding in hard soils does not commend itself.

Experiment XXVIII.

In this experiment four plots each 8×2 metres were treated thus.

I. Hand-weeded twice a week by the fork uprooting the basal bulb.
II. Untreated control.
III. Hoeing with Planet Junior Hoe twice a week.
IV. Hoeing with the Planet Junior Hoe once a week.

The plots were in the Empress Gardens. The adjacent ground was irrigated three times a month. The plots were not irrigated, but on February 15, water was accidentally allowed to flow over the plots.

A test digging on December 6 at the edge of all plots except the control plot gave the following results (Table XVII). Counts were made of the shoots before each weeding. Test diggings and their results are shown in Table XVIII. The first test digging was taken at the edge of the plots and the number of tubers in these diggings was probably due to invasion from neighbouring soil. The second test digging was taken

in the middle of the plots and the effect of the weeding and hoeing is apparent.

TABLE XVII.

Depth of digging	Opposite Plot I weeded. No. of tubers	Opposite Plot III No. of tubers	Opposite Plot IV No. of tubers
Surface to 6″ ..	437	517	491
6″ to 12″ ..	275 } 587	231	418
12″ to 18″ ..	312 }		
18″ to 24″ ..	75	19	174
24″ to 36″ ..	14	0	0
Total of tubers ..	1,113	767	1,083
Surface shoots ..	82	76	17

TABLE XVIII.

Test digging in the Empress Gardens plots in weeded and hoed areas. Taken on 15-6-23 *(in the middle) and on* 26-10-23 *(at the end) of the Experiment.*

Plot No.	Operation	Digging on dates	Surface shoots	Total tubers	Total of normal tubers	Total of empty tubers
1	Weeded	15-6-23	11	295	215	80
		26-10-23	1	213	17	196
2	Control	15-6-23	344	952	941	9
		26-10-23	235	813	790	23
3	Hoed twice a week ..	15-6-23	35	475	463	12
		26-10-23	5	190	19	171
4	Hoed once a week ..	15-6-23	28	454	448	6
		26-10-23	16	367	270	97
Digging in an area less infested than the control plot II ..		15- 6-23	40	280	271	9
		26-10-23	143	404	301	103

The net result again is this :—

Thorough weeding does reduce the number of shoots and tubers, but takes a long time and must be continuous.

By itself, weeding, even if very careful and continuous, can hardly be looked on as a method of extirpation in a badly infected area.

Experiments in the effect of cover :—

The next series of experiments deals with the effect of cover on the life of the *lavala* weed.

5. *Experiments in the effect of artificial cover.*

Experiment XXIX.

In Ganeshkhind Botanical Garden, in the vine plantation, where the ground had recently borne a cucurbit crop for the purpose of keeping down weeds, a plot 18×19 ft. was covered with bamboo matting on December 13, 1921. A similar plot was taken as a control. Test diggings showed no tubers in the second or third foot of soil in either case. In the first foot of soil in an area 3×3 ft. surface the number of tubers in the plot to be treated was 10 and in the control 15. This number is low, showing some effect probably of the cucurbit crop and previous cultivation. No water was given to either plot. On January 24, 13 shoots had pierced the matting. Several thicknesses of newspaper were therefore pasted over the matting. Once a month from February 1, onwards the plots were examined. The thirteen shoots became sickly, bent and shrivelled and no further germination occurred in either plot. Two test diggings in each plot were taken on September 7, 1922 after 11·78 inches of rain had fallen. The bamboo matting had to some extent kept out this rain. The control had a growth of other weeds, but neither showed lavala. The results of digging the usual 3×3 ft. pit were,

Covered plot. First foot 8 normal and 3 empty tubers,

Control, first foot 18 normal and 9 empty tubers.

This experiment was abandoned as a worse infested plot was required to give significant results. The only possible conclusion that can be drawn is that the cucurbit crop had adversely affected the tubers.

Experiment XXX.

On the College Farm an area 10×10 ft. was covered by bamboo matting on 13th December 1921. The area was isolated from the surrounding land by digging a trench round it 3 ft. deep and 1½ ft. broad. This incidentally

allowed us to get a measure of the density and distribution of the tubers in the soil of this part of the field. Table XIX shows this density and distribution. The plot was very badly infected and is in marked contrast to the Ganeshkhind Garden plot in the last experiment which we must consider as nearly free from the weed. On January 24, 1922, it was found that 23 shoots had pierced the matting. Several thicknesses of newspaper were then pasted over the matting. On February 10, shoots were appearing below the matting but were yellow and bent. On April 7 shoots were found issuing from the sides of the surrounding trench. The sides of the trench were then covered with matting. On May 4, it was found that shoots were piercing the matting on the sides of the trench. The trench was therefore filled up on that date. On September 1, one corner of the covered area measuring 1×1 ft. was dug out to a depth of 3 ft. No tubers were found in the third foot and a total of 100 tubers in the upper 2 ft. All were normal except those in the top 3 inches. These 100 tubers were planted in good soil at 3 ft. deep in Modi Bag and watered. By the end of September 40 had germinated. The period of covering (nine and a half months) was therefore clearly insufficient to kill the tubers beneath the cover.

TABLE XIX.

The number of tubers in the trenches around the area under cover of the matting.

Trench No.	Area of the trench	Shoots on the surface	TUBERS AT DIFFERENT DEPTHS			TOTAL
			In the first foot	In the second foot	In the third foot	
1	10′ × 1½′	335	730	420	40	1,525
2	,,	410	921	514	74	1,919
3	,,	380	678	284	56	1,398
4	,,	217	442	204	45	908

Another test digging of the same kind was done on February 27, 1923, and the tubers similarly tested for germination. From the 18 to 24 inches depth only six tubers were got and none of these germinated. Above 18 inches tubers were numerous (total 829, of which 462 were not empty) from each 6 inches layer 40 were taken and put to germinate. Between March 10 and April 23, tubers from the top 6 inches germinated to the extent of 36 out

of 40, tubers from the second 6 inches to the extent of 32 out of 40. In April 1923 an iron sheet 10×4 ft. was laid on the soil near the plot covered by matting. In June a test digging revealed the fact that almost all tubers were plump and healthy. The sides of the sheet were then earthed up. Nevertheless, throughout the rains, shoots were being produced beneath the iron sheet. These were naturally etiolated. When the area was finally dug up it was found that many of the original old tubers were empty. The lavala plants round about the covered area had sent in tubers under the iron sheet at about the six inches level. Table XX gives a comparison of the results as shown at the June test digging. The much reduced number of tubers in the middle of the matting cover shows that, at the side of the covered block of earth, air, light and infection from outside had all conspired to keep the tuber number high, while in the less favourable conditions in the centre of the matting the tubers were gradually being reduced. This, however, was after three months continuous cover.

TABLE XX.

Test diggings (3′×3′) *in the area covered with matting, College farm, June* 12, 1923.

Locality of the test digging	Date	Surface shoots	Tubers at different depths								Total of normal tubers
			0–6″		7″–12″		13″–18″		19″–24″		
			Empty	Normal	Empty	Normal	Empty	Normal	Empty	Normal	
Matting —											
N. W. Corner ..	12–6–23	0	155	556	105	109	112	201	2	10	876
S. W. Corner ..	,,	2	219	314	119	109	25	41	4	2	470
Centre	22–6–23	2	224	55	243	56	3	0	0	0	111
Under the iron sheet ..	12–6–23	149	0	315	12	240	1	120	0	17	1,192
Control from a harrowed plot	,,	12	15	739	0	221	3	107	0	11	1,078

The area, which had been under the bamboo matting, was exposed to the sun from July 27, 1923. Within a week shoots appeared on the surface near the edges. Three test diggings were made to ascertain the nature of the surviving tubers on August 8 and 14, 1923. Table XXI gives the results. The tubers from the third digging were transplanted to test viability. The

following is the result (Table XXII). The time of germination was from August 8 to October 31.

TABLE XXI.

Test diggings in the area under the matting cover, College Farm, Poona.

No. of pit	Date	Total of good tubers	Tubers in the different layers up to: —									
			6″		1′		1½′		2′		2½′	
			Normal	Empty	Normal	Empty	Normal	Empty	Normal	Empty	Normal	Empty
I	14-8-23	209	**30**	143	**81**	20	**47**	67	**34**	3	**8**	6
II	,,	127	**6**	14	**55**	113	**46**	69	**19**	61	**1**	9
III	8-8-23	95	**11**	36	**30**	23	**28**	10	**17**	8	**9**	4

TABLE XXII.

Germination of the transplanted tubers.

1	Tuber out of	11	from the	6″	depth.	9·9 per cent. Germination.
3	Do.	30	,, ,,	1′	,,	10·0 ,, ,, ,,
5	Do.	28	,, ,,	1½′	,,	18·9 ,, ,, ,,
8	Do.	17	,, ,,	2′	,,	47·0 ,, ,, ,,
0	Do.	9	,, ,,	2½′	,,	0·0 ,, ,, ,,

Later, on October 29 and 31, 1923, two dense patches of the weed that had arisen on the area after the removal of the bamboo matting were dug out and examined. The shoots were found to proceed from tubers below 18 inches deep. The upper tubers were dead. The total number of tubers was 247 (in the usual test digging of 3×3×3 ft.) of which 114 were empty, 23 normal solitary and 110 normal in chains. New tubers had been produced below 18 inches.

The general conclusion from these experiments is that cover is a very ineffective means of dealing with the tubers. Below 18 inches the original tubers may survive for more than two years and at that depth new tubers may be formed.

Experiment XXXI.

Pabco mulch is a specially prepared and waterproofed paper which is spread on land to keep down weeds and conserve moisture.

The paper was applied to land containing the *lavala* weed in the following ways :—

1. A square of 3 × 3 ft. was dug to three feet deep and cleaned of tubers. 100 tubers were then planted in the top six inches. Pabco mulch was laid on the top and firmly secured by earthing up the sides.

2. Another 3 × 3 ft. area was weeded so as to remove the surface shoots but leave those just about to appear. On this area the paper was laid.

3. In another 3 × 3 ft. area the paper was laid on the plants as they were. All these three plots were unirrigated.

4. A sheet of Pabco was laid on the earth of a water channel so as closely to cover the weeds and allow water to run over the paper.

5. An irrigated area 5 × 3 ft. was covered.

6. An area 20 × 3 ft. was covered and irrigated after laying down the paper.

These experiments were laid out on September 7, 1923. By September 17, 1923, in every one of these experiments the lavala shoots had pierced the paper, formed basal bulbs and developed normal aerial parts. Adult leaves which had been covered had suffered, but the spearheads of the new shoots were quite sufficiently rigid to penetrate the cover.

Experiment XXXII.

At the Government Farm, Surat, a plot 10×10 ft. showing 25 lavala shoots was covered by cut grass on February 21, 1922. This grass was allowed to stay on the ground till May 22, 1923. During this period the grass pile which was originally five feet high settled down to about three feet and received the monsoon rain, thereby becoming rotten. Lavala shoots managed to come through the edges of the cover. A small part of the cover was removed and a test digging made on August 18, 1922, and again when the grass was removed. (Table XXIII.)

TABLE XXIII.

Test diggings from	Date	Tubers	
		Normal	Empty
A. Covered area	Aug. 18, 1922	6	1
	May 22, 1923 (mean of three)	0	10
B. Control outside covered area ..	Aug. 18, 1922	43	35
	Dec. 29, 1922 (mean of three)	172	117
	May 22, 1923 (mean of three)	89	0

In this case the cover has certainly killed the tubers.

Experiment XXXIII.

At the Government Farm, Surat, near the area of the last experiment, another plot of 10×10 ft. was covered to a depth of six feet by cotton stubble. This pile settled down to about five feet and was penetrated at its edges by lavala shoots. The cover was removed on May 22, 1923.

In this case also all the tubers in three test diggings were empty.

In both experiments the top soil up to six inches deep from the surface beneath the cover had dried, but the layers below had a good deal of moisture.

Experiments in the effect of Agricultural Operations.

The next series of experiments deals with the effect of agricultural operations in the field on the weed.

Experiment XXXIV.

At the Government Fodder Farm, Nadiad, a plot 66×66 ft. was ploughed and harrowed, and the number of tubers brought to the surface counted. An idea of the original density of the tubers in this plot was got by two test diggings, of which the figures are in Table XXIV.

TABLE XXIV.

NADIAD.

Test Diggings before the operations.

I. PLOT TO BE PLOUGHED—(average of two test diggings).

	First foot	Second foot	TOTAL
Tubers in	222	43	265

II. CONTROL PLOT—(average of two test diggings).

	First foot	Second foot	TOTAL
Tubers in	191	13	204

All these are healthy tubers.

TABLE XXV.

Test Diggings after the operations.

I. PLOUGHED PLOT—(average of three test diggings).

	On surface	First foot	Second foot	TOTAL
* Tubers ..	52	147	104	303

II. CONTROL PLOT—(average of three test diggings).

	First foot	Second foot	TOTAL
Tubers in	48	75	123

The operations were as follows :—

December 14, 1921. Ploughing to seven inches deep with a BT2 plough.
December 16, 1921. Breaking of clods by hand with hammers.
December 20, 1921. Disc harrowing.
February 12, 1922. ,,
February 26, 1922. ,,
March 12, 1922. ,,
March 27, 1922. ,,
March 30, 1922. Second ploughing to seven inches with BT2 plough.
April 13, 1922. Disc harrowing.
April 17, 1922. ,,

The farm was closed in May, 1922.

During the experiment the total number of tubers brought to the surface in an area of 3×3 ft. (average of two such areas) was 170.

Final test diggings were taken on May 25, 1923, and are given in Table XXV.

The plot was revisited in August 1922. Lavala shoots were present in the plot and were found to be coming mainly from tubers in the second foot of soil.

* Tests showed that of the tubers on the surface all were dead and from the next six inches 70 per cent. were dead in the ploughed plot. This reduces the actual living tubers in the first plot to 203.

In this experiment, however, we can see that a greater number of test diggings should have been averaged to get reliable comparative results. What we do learn is that agricultural operations bring to the surface a great number of tubers and that these die.

6. *Experiments in the competition of other plants with* Cyperus rotundus.

The next set of experiments deal with the competition of other plants with *lavala*.

Experiment XXXV.

Effect of Grasses on Lavala.

Several observations indicated that it might be possible to smother *lavala* by grasses. Some such indications were as follows :—

(1) Near canals in fields where lavala was growing it was absent from patches of hariali grass (*Cynodon dactylon*).

(2) At Khandala, Lonavala and Karjat, lavala abounded in the rice fields but was absent from the adjoining grass lands.

(3) At Surat Farm an experiment in growing wild fodder grasses gave hopes of smothering lavala by this method.

(4) In Gujarat it is a well known practice to leave very badly lavala infested fields fallow for a number of years. Grass grows on such fields and the lavala dies out. The fields are then again taken into cultivation.

On the other hand, however, in the polo ground of the Willingdon Sports Club in Bombay and in the Gardens of the Bombay Improvement Trust, lavala was found growing amongst hariali grass in sufficient quantity to be regarded as a pest. In both cases it is believed that the tubers were brought in from the race course with horse manure stacked there,—the lavala being present in that area.

The following eight experiments were accordingly arranged :—

(1) Isolated tubers were planted on a lawn in the bungalow compound of Dr. Burns, Economic Botanist, Poona. This lawn was practically pure *Andropogon annulatus* and *A. caricosus*.

(2) Tubers were planted in grass areas adjoining the Karjat Rice Experiment Station.

(3) Tubers were planted in the lawns of the Improvement Trust Garden, Bombay. The grasses there were hariali (*Cynodon dactylon*) and heda (*Paspalum distichum*).

(4) Tubers were planted amongst heda grass in Modi Bag in the College of Agriculture, Poona, and heda grass was planted amongst lavala in the College Farm.

(5) The effect of natural invasion of hariali on a lavala plot was observed on the College Farm.

Experiment XXXVI.

Planting of isolated tubers in the Economic Botanist's lawn.

The lawn was divided up by paths radiating from the centre, into seven triangular sections. The lawn was flooded with canal water once in ten days except during the rains. One metre quadrat was taken in each sector and one big selected tuber planted three inches deep in the centre of each quadrat, on 19th August, 1922. By 28th August the tubers in five quadrats had germinated (1, 2, 3, 5 and 6). They did not, however, produce any more shoots or tubers up till 23rd November 1922. The grass was then cut. Fresh tubers were then planted in all the quadrats except the third and fifth where the shoots were still alive (the tubers in 1, 2 and 6 having died). On 31st April, 1923, the tubers of the second planting in quadrats 6 and 7 had one shoot each. All the others were dug out and found to be empty and dead. A third planting of tubers in the same quadrats was then done on 31st April, 1923.

On June 16, 1923, it was found that the tubers in quadrats 3 and 5 from the previous planting had produced one tuber each and had died and the tubers in 1, 2 and 6 had produced straggling shoots which were withering and the tubers in 4 and 7 were dry and shrivelled. A fourth planting was done after digging out these tubers on June 21, 1923, and five tubers were planted in each quadrat area. These had the benefit of the rains, but the grass also grew thick and an observation taken on October 29, 1923, shows that only one tuber had germinated out of the whole lot of 35 tubers.

This shows that the tubers were unable to produce more growth in a soil already full of grass roots and that the dense cover on the surface prevented vigorous aerial growth.

Experiment XXXVII.

Planting of tubers in grass areas adjoining the Karjat Rice Experiment Station.

Five quadrats were marked out on a slope full of grass on the west of the present office building at Karjat.

Quadrat I. Grass undisturbed (control plot).
,, II. 100 tubers from Poona fields were planted in a grass plot.
,, III. 100 tubers from Karjat rice fields were planted in a grass plot.
,, IV & V. 100 tubers were planted in a grass plot from which the grass had been weeded (IV and V were duplicates).

The tubers were planted in all the quadrats on 22nd August, 1922, and the conditions were favourable as rain was falling at the time.

The tubers from Poona were transported in wet gunny bag material and hence there was no fear of their drying out before planting.

These quadrats were again examined on 6th December, 1922. Observations :—

I. Grass normal.
II. Only seven tubers had germinated out of the 100. The shoots were weak.
III. Twelve tubers out of 100 had germinated.
IV. Fifty-four out of 100 had germinated.
V. Sixty-six out of 100 had germinated.

Out of these plots IV and V, however, with their total of 120 germinated tubers, only 30 shoots were green at the time of observation. Out of these again four only had produced small tuber colonies of 6, 3, 5 and 6 tubers respectively.

The quadrats were again observed on 6th April, 1923. Observations :—

I. Grass dry.
II. All the tubers shrivelled. They were dug out.
III. Three lavala plants were alive, of which two had four and two tubers respectively.
IV and V. Dried shoots of 20 plants were found in these two taken together, and in the soil 50 dormant healthy tubers. The living plants and the 50 dormant tubers were replaced in the same quadrats.

The quadrats were finally observed in August 1923. Observations :—

I. Grass thick.
II. Grass thick. No lavala visible.
III. Grass thick. No lavala visible.
IV and V. Grass thick. No trace was found of the plants and tubers replaced in these quadrats.

This preliminary experiment shows that in grass land on the slopes of this hill the soil conditions were unfavourable to this plant and the thick matting of the fibrous grass roots gave little scope for the lavala tubers to sprout, root and multiply. In the weeded plots the lavala got a footing and only the uprooting of the tubers stopped their growth.

Experiment XXXVIII.

Planting of tubers in the lawns of the Improvement Trust, Bombay.

Two quadrats in a hariali lawn were laid down and 50 *lavala* tubers planted on 24th August, 1922. At the same time 40 heda grass stumps were planted in the same quadrats, without removing the existing hariali. There were already at the time of planting on quadrat—

I. Lavala shoots 56.

II. ,, 49.

The lawn was mowed on 12th September, 1922, and 21st September, 1922, and on September 24, the position was—

Quadrat I. Lavala shoots 82. Heda stumps 40.

,, II. ,, ,, 65. ,, ,, 39.

The place was again observed on 6th December, 1922. The stumps were unaltered in number but were dry and stunted. The lavala shoots were (I) 30 and (II) 27 respectively. Hariali was growing thickly on both quadrats. On the same day both quadrats were dug out and the tubers were counted. Results :—

TABLE XXVI.

	Shoots	Tubers	
		9–12″	12–18″
(1) Control test digging at the time of planting (24–8–22)	60	120	80
(2) Control test digging at the time of digging out the quadrats (6–12–22)	30	189	100
(3) Quadrat I	30	150	50
(4) ,, II	27	139	25

The soil was one and a half feet deep over sand. The tubers dug out were all plump and dormant.

In this experiment there is evidence of a smothering effect by the grass especially in reducing the number of deep-lying tubers. This experiment was then discontinued. The control test diggings both on 24th August, 1922, and 6th December, 1922, were done on areas of the same surface size as the experimental quadrats and on ground near to them. These diggings indicate that the lavala although somewhat reduced was existing along with the hariali and that it was vigorous.

The fact that the lavala grew well along with the hariali may have been due to the fact that the lawn was comparatively newly made with imported earth.

In another quadrat (III) the soil was dug up to six inches deep and the original lavala and hariali removed on 24th August, 1922. Heda grass was planted on the same date in the plot along with 50 tubers of lavala. The grass grew vigorously. Out of the 50 tubers 30 had germinated by 6th December, 1922.

In still another quadrat (IV) 50 tubers were planted in a plot prepared like III by digging to six inches deep, but no grass was planted along with the tubers on 24th August, 1922. 42 out of the 50 germinated by 6th December, 1922. Both these quadrats were dug out on 6th April, 1923. The following is the result :—

TABLE XXVII.

	Shoots	Tubers	
		0–12″	12–18″
Quadrat III	35	35	10
,, IV	93	122	20

The soil in these two quadrats had been recently deposited and was underlaid by sand. Here the heda grass had had a distinct effect on the vigour of growth of the lavala though it had by no means checked it.

Experiment XXXIX.

Planting tubers in Modi Bay amongst grass.

On 19th September, 1922, eight metre-quadrats were prepared. These were sited in an unirrigated area already naturally infested with lavala. 100 heda stumps were planted in each quadrat. The number of tubers in the adjoining test digging were—

Shoots	First foot	Second foot
236	257	120

The quadrats were watered twice a week for about a month. Thereafter there was no irrigation. Table XXVIII shows the number of shoots of

lavala and of living heda stumps (at the time of planting and on November 23, 1922) :—

TABLE XXVIII.

Quadrat No.	LAVALA – SHOOTS		HEDA STUMPS	
	Dates		Dates	
	19–9–22	23–11–22	19–9–22	23–11–22
1	185	258	100	86
2	276	382	100	72
3	319	438	100	45
4	215	316	100	62
5	263	304	100	69
6	320	373	100	46
7	277	384	100	23
8	314	815	100	24

The heda stumps died for want of irrigation and lavala completely filled the quadrats.

On 19th September, 1922, 500 heda stumps were planted in two small strips 8 × 2 and 6×2 ft. at right angles to one another near a small tank in Modi Bag. This grass thoroughly established itself. On 11th October, 1922, 36 lavala tubers were planted 20 in one arm and 16 in the other arm of the L. They were planted at regular intervals at three inches deep. On 20th November, 1922, 47 lavala shoots were visible, 32 in the one arm and 15 in the other, and 525 heda stumps (342 and 183 respectively).

One lavala plant from near the side of the grass plot was dug out. It was found that the original tuber had produced one shoot and a chain of three tubers, growing horizontally. The last two tubers were just outside the grass patch and were sprouting. On 10th July, 1923, the plot had 75 shoots of lavala (40 and 35) and the heda clumps were very dense.

Both the strips were dug out in September, 1923, when the soil was workable. The heda clumps were very dense and only (30 and 20) 50 lavala shoots were seen on the surface. But of the original 56 tubers planted three inches deep only 35 were found to be sprouting at that time. The others were empty.

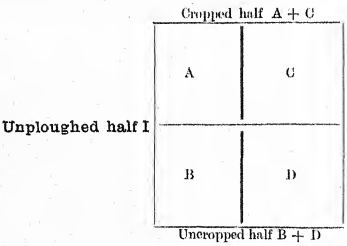
The rooting of the grass was not deeper than four inches. But chains of big tubers were found beneath the six inches layer lying horizontal between six to twelve inches, sometimes coming out the covered area and producing shoots. 106 such tubers were found.

We have here a clear indication of the tactics of the lavala plant in competition with other plants. The lavala struggles to put up an aerial connection, wherever possible. From tubers so connected it sends off droppers to layers unoccupied by the roots of the competing plants, there it stores food material and from them or from the original tubers it sends off horizontal rhizomes to explore for places outside the dense competing aerial cover where it may send up shoots more successfully.

Experiment XL.

7. *Experiments on the Effects of Cultivation and Cover Crops.*

The field chosen for this experiment was on the Government Farm, Surat, and carried a crop of jowar from June to November 1921. Test diggings of the usual dimensions (3×3×3 ft.) were taken before the start of our experiment and showed a dense tuber population. The plot was divided into two areas and the first was left untreated, while the second was submitted to the agricultural and cultural operations shown in table XXIX. These continued till August 1922, when in each area one half was sown with jowar and the other half left as a control. The following sketch will make the position clear.



The sowing of jowar on the first area meant that half of it had to be lightly ploughed before sowing.

Test diggings were taken throughout the experiment and these are recorded in table showing the changes in the tuber population. After the jowar was harvested, the original area II containing the C and D sub-plots was again thoroughly and continuously cultivated until the following June.

TABLE XXIX.

Combined Experiments at Surat on the Effect of Cultivation and a Cover Crop.

Date of test digging	Operations between test diggings	AREA I. TUBERS FOUND AT EACH TEST DIGGING		Operations between test diggings	AREA II. TUBERS FOUND AT EACH TEST DIGGING		REMARKS
		Normal	Empty		Normal	Empty	
12–12–21	None	471	0	None	546	0	Dense healthy tuber population in all plots
24–4–22	None	509	0	Gallows ploughed, Norwegian harrowed. Disc harrowed	405	10	A little improvement in the ploughed plot
30–5–22	None	175	0	Tooth cultivator worked	135	0	Marked drop in tuber population, of all plots, probably effect of hot weather; fewer tubers in ploughed plot
28–8–22	None	3	141	None	1	51	Deaths of tubers in all plots probably due to continued hot weather; fewer tubers in ploughed plots
		(Average two diggings)					

TABLE XXIX—*contd.*

Date of test digging	Operations between test diggings	AREA I. Tubers found at each test digging — A Normal	A Empty	B Normal	B Empty	Operations between test diggings	AREA II. Tubers found at each test digging — C Normal	C Empty	D Normal	D Empty	Remarks
30-10-22	Plot divided into equal parts. A & B both lightly ploughed and A sown with jowar on 28-8-22. B not sown. Crop standing at time of this test digging.	40	90	39	278	Plot divided into equal parts C & D. C sown with jowar, D not sown	11	221	25	25	Recovery of the plots weed in all lavala due to rain; Plots C & D (originally ploughed) together show a smaller total tuber population and a smaller percentage of living tubers than the unploughed A & B. The effect of the cover crop is not consistent in A & C
26-12-22	Harvesting crop (figures are average of three diggings)	16	64	32	108	Harvesting crop (figures are average of three diggings)	45	46	14	68	C & D still have a less total tuber population; but their percentage of living tubers is now greater than in the A & B plots. The effect of the cover crop is negligible

5-6-23	None (additional Test diggings at junction of A & B plots)	178 40 Normal 69	57 4 Empty 6	Gallows ploughing once: ploughing by Broach plough twice weekly (additional test digging at junction of C & D plots)	0 91 Normal 0	0	118 Empty 151	In A & B plots the lavala is rapidly regaining its hold. In C & D it is extirpated.

A study of the Table XXIX along with its remarks column brings out the following points.

(1) In both areas the lavala had a bad time in the hot weather of 1922, the rain, however, revived it considerably and until the crop was harvested we cannot say that there was a very striking difference between the area which had been ploughed in the preceding hot weather and that which had not, although there is undoubtedly a reduction of the tuber population in the area ploughed.

(2) The effect of the cover crop in both areas on reducing tuber production, taken against the control plot in its own area, is negligible. In fact the figures give a less tuber viability for the sub-plots without the cover crop.

(3) The cultivation in the second hot weather has, however, been absolutely effective as the figures amply show. The untreated area (both A and B) has a large tuber population (not however anything like equal to its first condition) with a large percentage of viable tubers, while the treated area (C and D) has not a single living tuber left.

This is the most conclusive proof we could have of the value of *continuous* thorough cultivation in the hot weather. It indicates also that the battle may not be won in the first hot weather cultivation but should be won for certain if the same process is continued for a second hot weather after one rains crop.

An experiment similar to the above in which only a harrow was worked was carried out. Mere harrowing proved entirely ineffective as the deeper tubers were untouched.

Experiment XLI.

At Manjri near Poona in the vicinity of our sugar station we leased a piece of land badly infested with lavala. This area was 300 by 200 ft. in size and was divided into five plots.

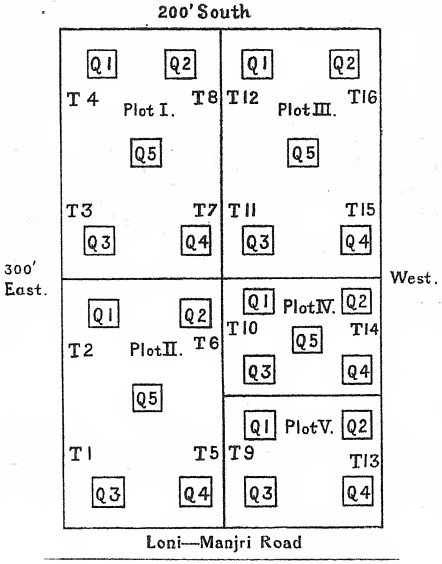
Plots I, II and III were sown to a cover crop, plot IV was an absolutely undisturbed control and plot V was ploughed once in the beginning.

Test diggings (3×3×3 ft.) were taken in each plot at the points marked T 1 to T 16.

The middle of the plot was worse infested than either edge. The eastern edge was probably affected by the shade and the roots of trees along that side. No tubers were found below 18 inches, the subsoil being shadu (chalky soil) or murum. The average distribution taking the average of all the diggings was 154 per digging of 3×3×3 ft., of the eight middle diggings 234, and of the eight diggings on the East and West edges 74.

Effect of cover on Lavala Plan.

Cover crop experiment at Manjri.



T. Test digging in the beginning of the experiment.
Q. Quadrat area on which the Lavala and sann plants are counted once every fortnight.
Plot I, II, III were under cover. Plot IV control untreated. V control ploughed once.

Treatment.

Plots I, II and III were ploughed in June 1923 by an iron plough. The rains were late. Sann hemp was sown at the rate of 30 lb. per plot of 150 by 100 ft. in July 1923. From August 1, weekly observations were taken to get some idea of the growth of the sann hemp and of the lavala. For this purpose five fixed quadrats were laid out (labelled Q1 to Q5) in each plot. The number of lavala shoots and sann hemp plants was counted in each quadrat. On September 4 and 5 irrigation was given as the sann was growing poorly, especially near the middle of plot I. Six additional quadrats were

also laid out in dense sann growth on September 15 and counted once. The sann hemp was cut and ploughed in on October 8, 1923.

Plate VIII (1) shows the ploughing in progress. The bunch of sann hemp held by the woman indicates the growth of the cover crop. Plate VIII (2) shows the lavala among the cut sann hemp. Such shoots had etiolated leaves from 8 to 12 inches long.

The total amount of sann hemp buried for plots I, II and III taken together was 5,700 lb.

Table XXX shows the results of the countings on the quadrats.

TABLE XXX.

Table of averages.

Plot No.	Average No. of lavala shoots per quadrat	Average No. of sann shoots per quadrat	Average height of sann (ft.)	Product of last two columns
I	153·8	133·2	3	399·6
II	90·0	104·0	2	208·0
III	110·2	164·2	3·5	574·7
IV	173·6	Not ploughed, no sann hemp		
V	17·2	Ploughed, no sann hemp		

Points to note :—

Where sann hemp is poor in growth lavala makes headway.

Ploughing and exposure by itself (plot V) has been more effective as to *shoot* reduction than sann hemp sowing.

The untreated plot IV was by far the worst.

When we come to consider tuber reduction, however, the sann hemp treatment easily wins. Table XXX(*a*).

TABLE XXX(*a*).

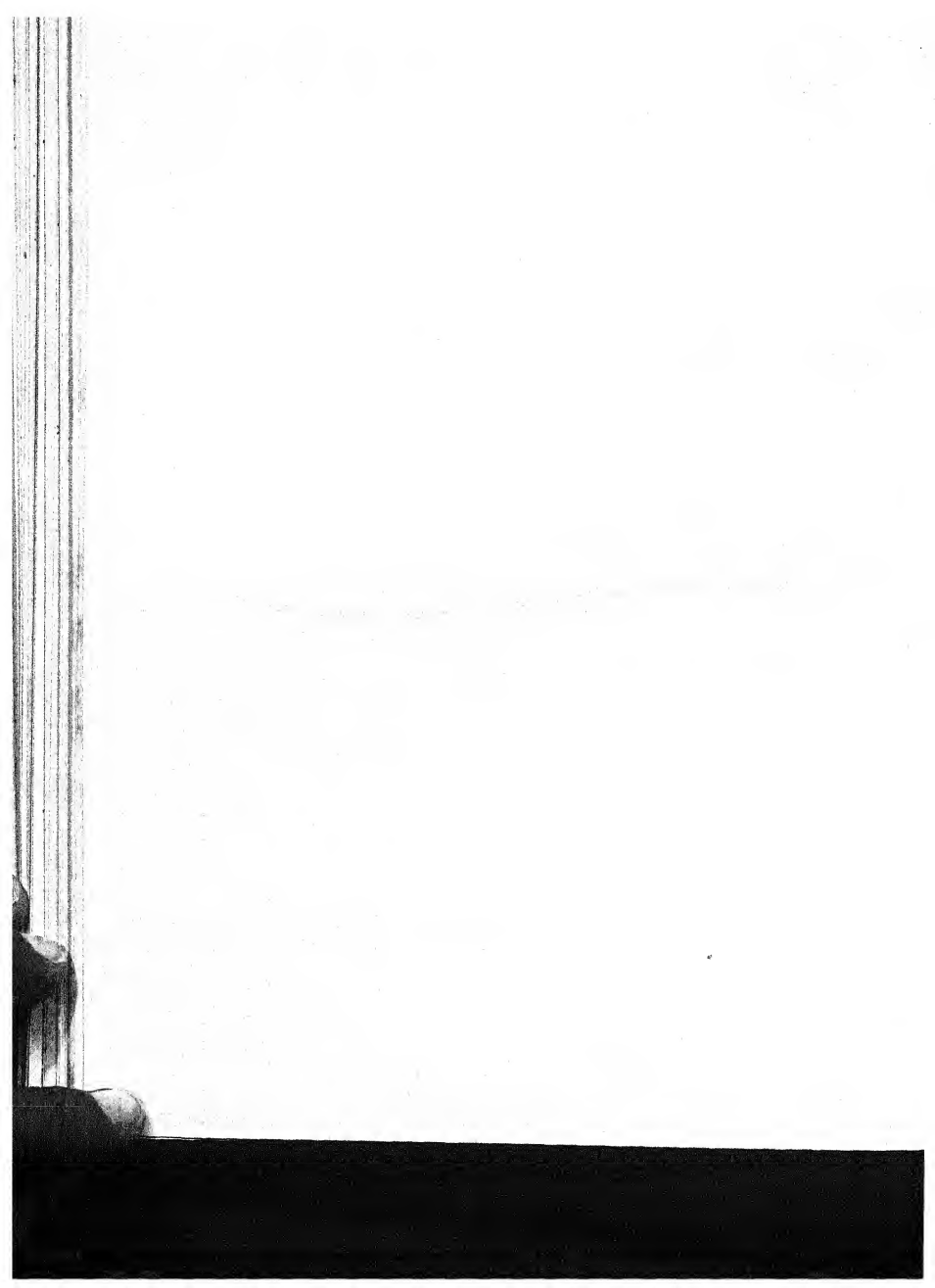
Effect of Sann hemp and ploughing on tuber population.

Plots I, II and III taken together (average of fifteen test diggings)	..	Number of tubers in 3×3×3 ft.
Before treatment	..	241 all sound
After treatment	..	160 of which 40 were dead
Plot IV (undisturbed control) average of five test diggings		
At start of experiment	..	251 all sound
At end of experiment	..	557 all sound
Plot V. (Ploughed control) average of five test diggings		
Before treatment	..	158 all sound
At end of experiment	..	228 all sound.

Ploughing and exposure in this case has *not* reduced the tuber population. The reason is that the exposure occurred in the rainy season.

1. Ploughing in progress. The bunch of sann hemp held by the woman indicates the growth of the cover crop.

2. Lavala among the cut sann hemp.



V. General Conclusions.

* "Hundreds of other opportunities could be suggested. Almost any insect or plant disease that is limited to a single host or presents a vulnerable point in its life history extending through a sufficiently long period of time to give the possibility of control may be considered as a possible candidate for eradication whenever a scientific investigation has progressed to such a point as to give the tools necessary for its accomplishment."

The above quotation can with equal truth be applied to weeds, and we propose now to examine how far the scientific investigations recorded in the preceding pages have provided the tools necessary for the eradication of the lavala weed.

The lavala weed, we have seen, is cosmopolitan in warm countries and is a weed of cultivation, particularly of irrigated fields. Salt and inundation destroy it, but as salt is injurious to other plants and inundation is important only in rice fields, these two methods of control can be left out of account at present.

The first important contribution to our equipment for eradication is the better understanding of the structure and life history of this weed. Let us briefly go over the main points established.

The plant as it occurs in the field is a colony of many tubers connected by underground rhizomes and distributed throughout the first three feet of soil. On the surface are its aerial shoots whose number bears no necessary relation to the number of subterranean tubers. Throughout the year and particularly in July and October, flowers are produced which result in the production of small fruit ("seeds"). The viability of these is exceedingly variable, from one to eighty per cent. according to circumstances. The number of seeds produced, however, is so enormous (calculated at fifty-four million seeds per acre), that even with a small germination percentage, seed production will obviously be an effective means of reproduction.

The prevention of seed production is therefore of importance.

The germination of the seed and development of the seedling have been studied. In this plant we have an extreme example of the geophilous habit,

* Research Work of the Department of Agriculture, by E. D. Ball, Director of Scientific Work, U. S. Department of Agriculture, in the *Chemical Age*, article copied in full in the *American Fertilizer*, Jan. 26, 1924, pp. 62–68.

whereby a plant immediately after establishing its first aerial connection devotes all its energy to producing a subterranean tuber or tuber-system, to escape climatic changes above ground. In the case of both seed and tuber germination and development we have found that *unless the seed or tuber can establish an aerial connection it cannot produce a tuber or tuber-system.*

The next indication of importance therefore is—

GERMINATING SEEDS OR TUBERS MUST BE PREVENTED FROM ESTABLISHING AN AERIAL CONNECTION.

If a seed or a tuber succeeds in establishing this aerial connection we find that it develops a "basal bulb" at the place where the aerial leaves join the stem and from this bulb in the case of the seedling or tuber-plant, and from the parent tuber as well, in the case of the latter, new rhizomes are then developed. In the seedling these rhizomes produce tubers, while in the case of the tuber plant they may form new aerial shoots as well as tubers. In many cases these new rhizomes are of the nature of "droppers," that is, positively geotropic rhizomes whose business it is to penetrate still deeper into the soil and there establish new bases more protected than the shallow-lying tubers. These tubers may in turn give rise to more rhizomes, and we soon have a tuber colony of considerable size.

The eradication of an established lavala colony is therefore a matter of very great difficulty. In the first place, in normal circumstances, all the tubers are viable. Drought may kill the superficially placed tubers, but the more deeply placed ones survive. Ordinary weeding touches only the very top tubers and normal ploughing and harrowing deals with the top foot of soil at the most. How then are we to deal with a thoroughly established colony ?

OBVIOUSLY, BADLY INFESTED LAND MUST BE CLEARED AT ALL COSTS AND THEN KEPT FROM FURTHER INFECTION.

The first step is the clearing.

In this connection we have studied the various methods for killing tubers. It has been found that although tubers can be found at three feet deep in the soil in a tuber system, isolated tubers buried at that depth or even at $2\frac{1}{2}$ ft. deep usually cannot produce a shoot that will reach the surface.

WE THEREFORE GET SOME HOPE THAT, IF ALL SUPERFICIALLY PLACED TUBERS CAN BE DESTROYED, AT LEAST SOME OF THE DEEPLY PLACED TUBERS WILL NOT BE ABLE TO PRODUCE SHOOTS WHICH WILL REACH THE SURFACE.

Again, theoretically, one would expect that the repeated removal of the aerial parts would result in the exhaustion of the reserve material in the tuber. In the case of isolated tubers this has been found possible, though even then the

resistance of the tuber to this depletion of its reserves is great. In a tuber-system the process of exhaustion, while undoubtedly taking place, is very much retarded by the fact that one is only dealing with a small proportion of the tubers and that the others are meanwhile either replenishing the tubers whose aerial parts are being attacked, or are themselves producing new aerial parts. All the same there is no doubt that—

REPEATED REMOVAL OF THE AERIAL PARTS AT SHORT INTERVALS FINALLY EXHAUSTS THE RESERVE MATERIAL OF THE TUBERS, which being translated into terms of practical agriculture means that we must be always weeding.

This mention of weeding brings us at once to the methods of weeding and to the consideration of their relative efficiency. Our experiments indicate that the least efficient is the method of the *khurpi* whereby merely the surface tuft of leaves is removed and the basal bulb (containing the growing point) is left undisturbed. Weeding which tears up this basal bulb is much more effective, but is at the same time only possible by a hand implement and at the expense of much time. Planet Junior Hoe weeding never completely cleared the ground although carried on for many months.

IT IS APPARENT THAT BY ITSELF THE WEEDING OF THE SURFACE SHOOTS IS A VERY SLOW AND UNCERTAIN MEANS OF REDUCING THE TUBER POPULATION.

A more direct attack on the tubers was, therefore, necessary. The best method tried was the exposure of tubers to the sun's heat. The results so got were confirmed by laboratory experiments. The tubers are very resistant to mere oven-drying, but sunlight and sun-heat are very effective agents. The most hopeful sign we have got in the whole research is that—

TUBERS EXPOSED ON THE SURFACE OF DRY SOIL OR NOT DEEPER THAN THREE INCHES IN DRY SOIL ARE KILLED IN EIGHT DAYS, IF EXPOSURE TAKES PLACE IN THE HOT WEATHER.

This indicates to us, therefore, that we must devise methods for bringing to the surface the maximum number of tubers for the maximum length of time at the hottest part of the year. The practical success of this method was clearly shown in the Surat ploughing experiment (Pages 171—173) where every tuber in the test diggings was dead.

Another methods of dealing with the tubers was cover of various kinds. It was found that matting and iron sheets were not effective and Pabco Mulch (a paper covering) entirely ineffective. Covering to a depth of several feet by grass or cotton stubble was effective but can hardly be regarded as feasible over large areas. The effects of a cover crop of jowar were negligible, but *sann hemp distinctly reduced the tuber population.* In all experiments, however,

the paramount importance of cultivation and particularly of continuous hot weather cultivation makes itself strikingly evident.

In fine, the results reduce themselves to this—

THE WEED MUST BE ATTACKED IMMEDIATELY AFTER THE RAINS AS SOON AS THE SOIL IS WORKABLE. THE PROCESS SHOULD INCLUDE DEEP PLOUGHING AND A SUBSEQUENT PULVERISATION AND TURNING OVER AND OVER OF THE BROKEN SOIL IN ORDER TO EXPOSE AS MANY TUBERS AS POSSIBLE TO THE SUN.

IF THE LAND IS NOT WANTED FOR A CROP IN THE FOLLOWING RAINS THEN A THICK GREEN MANURE CROP SHOULD BE GROWN AND PLOUGHED IN EARLY, FOLLOWING UP BY A SECOND DRY SEASON'S CONTINUOUS CULTIVATION. THE LAND SHOULD THEN BE CLEAR OF WEED.

IF THE LAND IS WANTED FOR A RAINS CROP, THE CROP SHOULD BE SOWN SO THAT INTERCULTIVATION CAN BE CONTINUOUSLY PRACTISED TO KEEP DOWN THE LAVALA SHOOTS AND PREVENT THE ESTABLISHMENT OF THAT AERIAL CONNECTION WITHOUT WHICH TUBER FORMATION IS IMPOSSIBLE. A SECOND DRY SEASON'S CULTIVATION SHOULD THEN REDUCE THE WEED, AND A THIRD SEASON'S WORK KILL IT.

Thereafter, there should be no difficulty in keeping the clean land from further infection.

APPENDIX.

Incubator experiment.

Table XXXI.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 93·2°F. (34°C.).

Date of heating, 10*th August,* 1921.

Table of weights (grammes).

	Duration of heating				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	9·25	8·01	10·742	8·272	8·072
Weight of 10 tubers after heating	8·88	7·80	10·380	8·000	7·760
Loss in weight	−0·37	−0·21	−0·362	−0·272	−0·312
Percentage of loss in weight ..	−4·00	−2·62	−3·460	−3·300	−3·860
Placed in a wet soil for 24 hours					
Weight after absorption of water ..	9·945	8·481	11·350	8·65	8·390
Change in original weight ..	+0·695	+0·471	+0·608	+0·37	+0·318
Change in heated weight					
Date of planting in soil two inches deep and watered ..	16th August, 1921				
Observations regarding germination	Began to germinate from 20th August, 1921				
No. of tubers germinated ..	9	8	8	9	7
Percentage of germination ..	90	80	80	90	70

INCUBATOR EXPERIMENT.

TABLE XXXII.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 98·6°F. (37°C.).

Date of heating, 9th August, 1921.

Table of weights (grammes).

	Duration of heating				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	9·74	10·96	9·94	8·640	9·57
Weight of 10 tubers after heating	9·45	10·68	9·78	8·355	8·33
Loss in weight	−0·29	−0·28	−0·16	−0·285	−1·24
Percentage of loss in weight ..	−2·90	−2·50	−1·60	−3·200	−12·90
Placed in a wet blotting paper for 24 hours					
Weight after absorption of water ..	8·40	11·645	11·195	8·895	10·00
Change in original weight ..	**−1·34**	+0·685	+1·255	+0·255	+0·43
Change in heated weight					
Date of planting in soil two inches deep and watered ..		16th August, 1921			
Observations regarding germination		Began to germinate from 20th August, 1921			
No. of tubers germinated ..	0	7	6	7	6
Percentage of germination ..	0	70	60	70	60
	These tubers had failed to regain the weight lost				

INCUBATOR EXPERIMENT.

TABLE XXXIII.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 104°F. (40°C.).

Date of heating, 4th August, 1921.

Table of weights (grammes).

	Duration of heating				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	10·045	9·991	9·750	9·82	10·670
Weight of 10 tubers after heating	9·750	9·702	9·527	9·53	9·352
Loss in weight	−0·295	−0·289	−0·223	−0·29	−1·318
Percentage of loss in weight ..	−2·090	−2·900	−2·280	−2·90	−12·300
	Placed in wet blotting paper for 24 hours				
Weight after absorption of water ..	10·428	12·208	10·003	10·426	10·876
Change in original weight ..	+0·383	+2·217	+0·253	+0·606	+0·206
Change in heated weight ..	+0·678	+2·506	+0·476	+0·896	+1·524
Date of planting in soil two inches deep and watered		11th August, 1921			
Observation regarding germination	Began to germinate from 20th August, 1921				
No. of tubers germinated ..	9	7	6	9	7
Percentage of germination ..	90	70	60	90	70

INCUBATOR EXPERIMENT.

TABLE XXXIV.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 110°F. (43°C.).

Date of heating, 3rd August, 1923.

Table of weights (grammes).

	Duration of heating				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	10·45	9·580	10·642	9·750	8·950
Weight of 10 tubers after heating	10·16	9·372	10·295	9·457	8·617
Loss in weight	−0·29	−0·208	−0·347	−0·293	−0·353
Percentage of loss in weight ..	−2·77	−2·190	−3·260	−3·006	−3·800
	Placed in wet blotting paper for 24 hours				
Weight after absorption of water ..	10·76	10·506	10·980	9·330	10·490
Change in original weight ..	+0·31	+0·926	+0·338	+0·420	+1·540
Change in heated weight ..	+0·60	+1·134	+0·685	+0·127	+1·773
Date of planting in soil two inches deep and watered		10th August, 1923			
Observation regarding germination	Began to germinate from 20th August, 1923				
No. of tubers germinated ..	8	8	3	7	8
Percentage of germination ..	80	80	30	70	80

INCUBATOR EXPERIMENT.

TABLE XXXV.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 112·8°F. (46°C.).

Date of heating, 2nd August, 1921.

Table of weights (grammes).

	Duration of heating				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	7·170	8·245	9·802	6·207	7·6
Weight of 10 tubers after heating	6·924	7·540	8·650	5·924	7·2
Loss in weight	−0·246	−0·705	−1·152	−0·283	−0·4
Percentage of loss in weight ..	−3·400	−8·500	−11·800	−4·500	−5·2
	Placed in a wet blotting paper for 24 hours				
Weight after absorption of water ..	7·560	8·600	9·380	6·710	7·76
Change in original weight ..	+0·390	+0·355	**−0·422**	+0·503	+0·16
Change in heated weight ..	+0·636	+1·060	+0·730	+0·786	+0·56
Date of planting in soil two inches deep and watered ..	..	9th August, 1921			
Observations regarding germination	Began to germinate by 20th August, 1921				
No. of tuber germinated ..	6	8	7	10	8
Percentage of germination ..	60	80	70	100	80

INCUBATOR EXPERIMENT.

TABLE XXXVI.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 120°F. (49°C.).

Date of heating, 5th August, 1921.

Table of weights (grammes).

	DURATION OF HEATING				
	10 Minutes	20 Minutes	30 Minutes	1 Hour	2 Hours
Weight of 10 tubers before heating	9·47	10·67	9·570	9·128	10·450
Weight of 10 tubers after heating	9·20	10·40	9·255	8·820	9·970
Loss in weight	−0·27	−0·27	−0·315	−0·308	−0·480
Percentage of loss in weight ..	−2·90	−2·50	−3·300	−3·300	−4·590
	Placed in a wet blotting paper for 24 hours				
Weight after absorption of water ..	10·155	11·595	9·880	8·980	10·72
Change in original weight ..	+0·685	+0·925	+0·310	**−0·148**	+0·27
Change in heated weight ..	+0·955	+1·195	+0·425	**−0·160**	+0·75
Date of planting in soil two inches deep and watered	..	12th August, 1921			
Observations regarding germination	Began to germinate from 20th August, 1921				
No. of tubers germinated ..	10	9	8	8	7
Percentage of germination ..	100	90	80	80	70

INCUBATOR EXPERIMENT.

TABLE XXXVII.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 112·8°F. (46°C.).

Date of heating, 2nd August, 1921.

Table of weights (grammes).

	DURATION OF HEATING (HOURS)				
	3	4	5	6	7
Weight of 10 tubers before heating	7·605	7·105	7·598	6·675	7·075
Weight of 10 tubers after heating ..	6·790	6·750	6·855	6·015	6·060
Loss in weight	−0·815	−0·355	−0·743	−0·660	−1·015
Percentage of loss in weight ..	−10·710	−4·990	−9·790	−9·890	−14·340
	Placed in a wet blotting paper for 24 hours				
Weight after absorption of water ..	7·265	7·145	7·385	6·384	6·605
Change in original weight ..	**−0·340**	**0·040**	**−0·213**	**−0·291**	**−0·470**
Change in heated weight ..	+0·475	+0·395	+0·530	+0·369	+0·545
Date of planting in soil two inches deep and watered			16th August, 1921		
Observations regarding germination	Began to germinate from 20th August, 1921				
No. of tubers germinated ..	4	0	2	1	1
Percentage of germination ..	40	0	20	10	10

INCUBATOR EXPERIMENT.

TABLE XXXVIII.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 120°F. (49°C.).

Date of heating, 11th August, 1921.

Table of weights (grammes).

	DURATION OF HEATING (HOURS)				
	3	4	5	6	7
Weight of 10 tubers before heating	7·550	8·628	10·940	7·798	8·573
Weight of 10 tubers after heating	6·965	7·982	9·884	7·059	7·340
Loss in weight	−0·585	−0·646	−1·056	−0·739	−1·233
Percentage of loss in weight ..	−7·740	−7·490	−9·660	−9·490	−16·800
	Placed in a wet blotting paper for 24 hours				
Weight after absorption of water ..	7·770	8·745	10·825	7·870	8·340
Change in original weight ..	+0·220	+0·117	**−0·115**	+0·072	**−0·233**
Change in heated weight ..	+0·805	+0·763	+0·941	+0·811	+1·000
Date of planting in soil two inches deep and watered		16th August, 1921			
Observation regarding germination	Began to germinate from 20th August, 1921				
No. of tubers germinated ..	7	5	3	6	6
Percentage of germination ..	70	50	30	60	60

INCUBATOR EXPERIMENT.

TABLE XXXIX.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 104°F. (40°C.).

A. TUBERS PLACED IN PAPER DISHES WITHOUT EARTH.

Date of starting, 20th March, 1923.

Table of weights (grammes).

	Duration of heating (hours)			
	3 I A	7 II A	8 III A	14 IV A
Weight of 10 tubers before heating ..	19·515	15·540	15·325	15·335
Weight of 10 tubers after heating ..	15·900	10·210	9·610	8·780
Loss in weight	−3·615	−5·325	−5·715	−7·555
Percentage of loss in weight ..	−18·520	−34·260	−37·290	−49·200
	Placed in a wet blotting paper for 24 hours			
Date	23–3–23	27–3–23	28–3–23	4–4–23
Weight after absorption of water ..	18·130	12·42	12·200	10·800
Change in original weight ..	**−1·385**	**−3·12**	**−3·125**	**−4·535**
Change in heated weight ..	+2·230	+2·21	+2·590	+20·200
Date of planting in soil two inches deep and watered	24–3–23	28–3–23	29–3–23	5–4–23
Observations regarding germination	Some germinated by 29th March, 1923, remaining observed till 2nd July, 1923.			
No. of tubers germinated ..	9	7	8	0
Percentage of germination ..	90	70	80	0

INCUBATOR EXPERIMENT.

TABLE XL.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 104°F. (40°C.).

B. TUBERS BURIED IN DRY EARTH.

Ten tubers are placed in a paper dish each heated for a definite time.

Date of starting, 20th March, 1923.

Table of weights (grammes).

	Duration of heating (days)			
	3 I B	7 II B	8 III B	14 IV B
Weight of 10 tubers before heating ..	19·15	16·84	16·35	19·01
Weight of 10 tubers after heating ..	15·22	10·36	9·63	11·29
Loss in weight	−3·93	−6·48	−6·72	−7·72
Percentage of loss in weight ..	−20·52	−38·47	−41·71	−40·61
	Placed in a wet blotting paper for 24 hours			
Date	23–3–23	27–3–23	28–3–23	4–4–23
Weight after absorption of water ..	17·920	12·71	12·35	13·30
Change in original weight ..	**−1·225**	**−4·13**	**−4·00**	**−5·71**
Change in heated weight ..	+2·700	+2·35	+2·72	+2·01
Date of planting in soil two inches deep and watered daily ..	24–3–23	28–3–23	29–3–23	5–4–23
Observations regarding germination	Some germinated by 29th March, 1923, remaining observed till 2nd July, 1923.			
No. of tubers germinated ..	10	8	8	0
Percentage of germination ..	100	80	80	0

INCUBATOR EXPERIMENT.

TABLE XLI.

Experiment of heating the tubers in paper dishes in an incubator at a constant temperature of 104°F. (40°C.).

A. IN PAPER DISHES WITHOUT EARTH.

Date of starting, 5th April, 1923.

Table of weights (grammes).

	DURATION OF HEATING — 15 DAYS IN ALL CASES			
	I A	II A	III A	IV A
Weight of 10 tubers before heating..	10·50	10·82	12·10	10·60
Weight of 10 tubers after heating ..	6·10	6·89	7·10	6·88
Loss in weight	−4·40	−3·93	−5·00	−3·72
Percentage of loss in weight ..	−41·90	−36·31	−41·32	−35·09
Average		39·665		
	Placed in a wet blotting paper for 24 hours on 20th April, 1923.			
Weight after absorption of water ..	6·63	7·73	7·32	7·52
Change in original weight ..	−3·87	−3·09	−4·88	−3·08
Change in heated weight ..	+0·53	+0·84	+0·22	+0·74
Date of planting in soil two inches deep and watered daily ..		21st April, 1923		
Observations regarding germination	Observed till 2nd July, 1923. Only one germinated on 1st May, 1923.			
No. of tubers germinated ..	0	0	1	0
Percentage of germination ..	0	0	10	0

INCUBATOR EXPERIMENT.

TABLE XLII.

Experiment of heating tubers in paper dishes in an incubator at a constant temperature of 104°F. (40°C.).

B. TUBERS BURIED IN DRY EARTH.

Date of starting, 5th April, 1923.

Table of weights (grammes).

	DURATION OF HEATING — 15 DAYS IN ALL CASES			
	I B	II B	III B	IV B
Weight of 10 tubers before heating ..	10·51	10·70	10·36	9·78
Weight of 10 tubers after heating ..	6·30	5·92	6·24	5·90
Loss in weight	−4·21	−4·78	−4·12	−3·88
Percentage of loss in weight ..	−40·05	−44·70	−39·77	−39·69
Average		41·052		
	Placed in a wet blotting paper for 24 hours on 20th April, 1923.			
Weight after absorption of water ..	7·00	6·30	6·71	6·30
Change in original weight ..	**−3·51**	**−4·40**	**−3·65**	**−3·48**
Change in heated weight ..	+0·70	+0·38	+0·47	+0·40
Date of planting in soil two inches deep and watered		21st April, 1923.		
Observation regarding germination	Observed till 2nd July, 1923. No germination			
No. of tubers germinated ..	0	0	0	0
Percentage of germination ..	0	0	0	0

STUDIES IN DISEASES OF THE JUTE PLANT.

(2) *MACROPHOMA CORCHORI* SAW.

BY

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In Eastern Bengal and Bihar, the jute plant (*Corchorus capsularis*) is sometimes attacked by a stem rot. The disease generally attacks the stem about the ground level or slightly higher, and in the early stages produces a pale reddish brown discoloration. This gradually darkens and spreads up and round the stem until the whole plant withers. Sporadic cases of this stem rot can be found in almost every field of jute, but it is only under certain conditions of cultivation that the disease becomes epidemic.

The disease may attack jute at any stage of its growth and occurs on both *Corchorus capsularis* and *C. olitorius*. Very young seedlings, about 2 in. high, die with the symptoms of "damping off," the hypocotyl becoming soft and completely rotted. In such specimens an abundant mycelium is found growing in the tissues of the young stem. On more mature plants the mycelial growth is accompanied by a copious formation of small black sclerotia in the interior of the stem among the bast fibres, and by the development of pycnidia which are visible as small black dots just below the cuticle.

The fungus

Examination of a diseased stem shows that the fungus consists of brownish coloured hyphæ and dark black sclerotia. Both hyphæ and sclerotia occur intermingled with the bast fibres. On the surface of the stem a number of minute black pycnidia can be seen. These pycnidia bear a close resemblance

to the sclerotia but can be distinguished by the presence of an ostiolum and by their more regularly spherical outline. At first sight the pycnidia and spores resemble those of *Diplodia Corchori* Syd.[1] in an immature stage. The pycnidia however are generally smaller than those of *D. Corchori* Syd., and the spores differ slightly in shape and in their proportions from the immature spores of *D. Corchori* Syd. The spore measurements are within the limits 16–32μ $\times$5–10μ and generally the width is about $\frac{1}{3}$ of the length (cf. *D. Corchori* 20–25μ $\times$ 10–13μ). Spores are unicellular hyaline and oval, sometimes slightly bent. Pycnidia are roughly circular in outline usually about 100–200μ in diameter, but some reach 260μ in diameter. The morphological characters of the fungus agree closely with those of a parasite of jute described recently by Sawada[2] in Japan and identified by him as *Macrophoma Corchori* Saw. nov. spec., and a comparison of material from India and Japan in both countries has confirmed the identity of the fungus with *M. Corchori* Saw.

The fungus was first obtained in culture by picking out a small piece of bast fibre, bearing sclerotia, from a diseased stem and placing it on the surface of a glucose agar slant. The fungus grew rapidly and pure cultures were readily obtained. On glucose agar the fungus produces a greyish white mycelium and small black sclerotia within the matrix. Fertile pycnidia with spores have never been obtained in artificial culture on any media. They appear to occur only on the living jute plant. Cultures on sterilized dead jute stems, and on decoctions of jute stems, all failed to produce pycnidia, nor has the pycnidium ever been found on any of the other hosts (e.g., cotton, potato) on which this fungus is parasitic. The sclerotia resemble closely the fertile pycnidia but are solid structures of less regular outline and of course lack the ostiolum. On the jute plant they are circular or oval in form and measure from 50μ–115μ in diameter. In culture they are sometimes much larger, reaching 168μ; such large sclerotia appear to result from the coalescence of several sclerotia.

Inoculations.

(1) Owing to the absence of pycnidia in cultures the connection of the pycnidium with the sclerotia and hyphæ was not at first suspected. In September 1917, six large jute plants on Pusa Farm, each about 8–10 feet high, were infected from a pure culture on glucose agar. Each plant was wounded

[1] Sydow, H. et P., et Butler, E. J. Fungi Indiæ Orientalis, Pars. V. *Annales Mycologici*, Vol. XIV, 1916, p. 196. Shaw, F. J. F. *Diplodia Corchori* Syd. *Mem. Dept. Agri. India, Bot. Ser.*, Vol. XI, No. 2, 1921.

[2] *Agri. Exp. St. Govt. of Formosa Bull.* 107, Nov. 1916 (Japanese). *Mycologia*, Vol. XI, p. 82, 1919.

by making a slight tangential cut on the stem surface at the point of infection. The infections were tied up with cloth. All the infections succeeded and a light brown discoloration spread up and down and round the stem, gradually darkening. The plants all withered and died with a copious development of sclerotia in the tissues and pycnidia at the surface. The pycnidia and spores were in agreement with those described above and the measurements of spores were within the limits 16–27μ × 6–8μ. The pycnidia were 160–200μ in diameter.

The presence of this pycnidium led to a fresh examination of the jute stem from which the culture used in the inoculation had originally been isolated. Here also pycnidia and spores were found similar to those developed on the infected plants. The spore measurements in this case were within the limits 17–29μ × 6–8μ and the pycnidia were 120–200μ in diameter.

(2) In 1918 a fresh isolation was made from sclerotia on a diseased jute plant on the Dacca Farm. This culture gave a normal growth of hyphæ and sclerotia, and from it four jute plants were infected on the Pusa Farm as in the previous experiment. Three of the infected plants died with typical symptoms of the disease and an abundant formation of pycnidia on the surface of the stem and of sclerotia and hyphæ in the interior. The pycnidia and spores were of the same size and shape as in the last experiment. The spore measurements were 16–24μ × 7–8μ.

From these infected plants spores were obtained in sterile water and plated on glucose agar. Single spores were then picked out, as soon as the agar had set, and transferred to glucose agar slants. In some plates the spores were left for 12 hours and then transferred to slants after they had germinated. In all cases pure cultures from single spores produced hyphæ and sclerotia—pycnidia and spores were never developed in culture.

Sclerotia were then picked out of one of the infected plants, washed in sterile water and plated on agar. From a plate single sclerotia were infected on glucose agar slants and some plates were left for the sclerotia to germinate. In all cases cultures precisely similar to those isolated from spores were obtained.

It follows from this that the pycnidium is a part of the sclerotial fungus and is an asexual spore stage of the same. An attempt was then made to isolate the fungus from spores and sclerotia obtained from one of the plants infected in 1917 (Experiment 1). The spores, however, failed to germinate but sclerotia germinated and gave normal cultures.

A further proof of the genetic connection between the sclerotia and pycnidia was afforded by a number of diseased plants collected from the

Dacca Farm. Among ten diseased plants, which all showed numerous sclerotia in the diseased tissues, two had pycnidia (180–200μ diam.) with spores measuring 20–27μ × 7–9μ. Single spore cultures and single sclerotial cultures from these plants were both normal.

(3) A culture was obtained from Poona and infected upon four wounded jute plants on 7th August, 1919. The parent culture had been isolated from potato and exactly resembled those obtained from jute in Pusa and Dacca. All the infected plants died with the development of pycnidia and spores of the usual type and measurements (16–27μ × 7–11μ). Single spore isolations from these plants developed the sclerotial stage of the fungus in cultures as in the previous cases. Another series of infections on jute from a culture isolated from a diseased cotton plant gave similar results. The infected jute plants developed the pycnidial stage of the fungus and isolations from spores produced the sclerotial stage in culture.

(4) In March 1921, a culture was isolated from diseased jute seedlings growing in Dacca soil. Six mature jute plants were wounded and infected from this culture on 22nd June, 1921. Two plants developed pycnidia and spores and the fungus was isolated from single spores as before.

(5) In July and August 1921, twelve jute plants on the Dacca Farm were inoculated from the same culture used in the last experiment. Five of the plants died with the development of pycnidia. Single spore isolations from these plants developed the sclerotial stage of the fungus in culture. During the progress of this experiment further isolations of the fungus from single spores, obtained from plants naturally diseased in the field, were carried out and confirmed previous results.

(6) In July 1923, a number of diseased jute plants were collected on the Dacca Farm. The diseased plants all showed pycnidia and sclerotia. The size of the spore on these plants was 16–24μ × 8–11μ. Spores were collected in sterile water and plated in the usual way. Single spore cultures all produced the normal sclerotial stage of the fungus. Six jute plants growing in pots at Pusa were infected from these cultures. Five of these plants died within 8 days of inoculation with a copious production of sclerotia and pycnidia.

The results of the inoculations therefore show that the pycnidial and sclerotial forms belong to the same fungus. The pycnidium occurs only on the jute plant and has never been obtained in artificial culture. The sclerotial form occurs in artificial culture and on jute, cotton, potato and other hosts. Cultures isolated from jute, cotton or potato give rise to the pycnidial form when infected upon jute.

Remedial measures.

Sawada[1] states that manuring with wood ash is very successful in lessening the incidence of the disease. This author considers that such treatment not only supplies the potassium which is necessary for the growth of the jute but also kills the spores of the fungus in the soil. Wood ash, applied at the rate of about 160 lb. per acre, is stated to have reduced the loss from this disease from 51 to 6 per cent. Liming the soil, according to Sawada, lessens the amount of disease, but heavy applications of nitrogenous manures favour the development of the disease.

These results agree with those obtained independently in India by Finlow[2], who found that on the Dacca Farm jute plots which had received potash were freer from disease than those to which potash had not been applied. In an experiment on the Dacca Farm the percentage of diseased plants in plots which had not been manured with potash ranged from 6 to 12 per cent. On plots which had received potash at the rate of 200 lb. potash (K_2O) per acre, the percentage of diseased plants ranged from $\frac{1}{2}$ to $1\frac{1}{2}$ per cent. In the potash-manured plots the yield of fibre was more than double that in the control plots.

Diseases of which the incidence is largely determined by the composition of the plant food present in the soil afford problems of great physiological interest. The deciding constituent may influence both parasite and host. Experiments now in progress at Pusa indicate that the fungus *Macrophoma Corchori* can produce a vigorous growth of mycelium on a culture medium which does not contain potassium. The field experiments at Dacca, however, suggest that jute is a crop which produces its heaviest growth when the amount of potash present in the soil is in excess of that normally required by other crops. It may well be, therefore, that a slight deficiency in the potash content of the soil would depress the vital activities of the jute plant to a much greater extent than it would affect the metabolism of the fungus, which is also living in the soil, thus rendering the host a more easy victim for the parasite. This aspect of the disease will form the subject of further research.

Macrophoma Corchori and Sclerotium bataticola.

The fungus *Macrophoma Corchori* Saw., which forms the subject of the present paper, produces its pycnidial stage only on the living jute stem while the sclerotia occur in culture and among the bast fibres of the jute stem.

[1] Sawada. *Loc. cit.*, p. 2.

[2] Finlow, R. S. *Rhizoctonia* in jute: The inhibiting effect of potash manuring. *Agri. Jour. India*, Special Indian Science Congress Number, 1918.

Sawada states that, in culture on agar media, immature pycnidia are formed. These immature pycnidia are evidently the same as the structures which have been termed sclerotia in the present paper. This sclerotial form of the fungus occurs also upon potato tubers where it causes a blackening of the eyes and a slight rot of the tissues, and also upon cotton plants. A certain proportion of the wilting of mature cotton plants and the damping off of seedling cotton appears to be due to this fungus. Upon these hosts the pycnidial form does not occur but infections with cultures isolated from these hosts on to jute have produced the pycnidial form on the latter host.

In the first investigations[1] on the parasitism of this fungus on jute, the connection of the sclerotial form, produced in culture, with the pycnidial form occurring only on the jute plant was not traced and the fungus was identified as *Rhizoctonia Solani* Kühn. In 1913, Taubenhaus[2] described a disease, Charcoal Rot, of the Sweet Potato which he attributed to a new species of the genus *Sclerotium* and named *S. bataticola*. Later, in 1917, Martin[3] described a fruit rot of pepper (*Capsicum annum*) as being caused by *S. bataticola*. It was then evident from a comparison of the illustrations of this paper with those of the earlier publication in India that there were strong points of similarity between the fungus identified in India as *Rhizoctonia Solani* Kühn and that subsequently named *Sclerotium bataticola* in America.

Cultures of the Indian fungus were submitted for examination in America and were pronounced by Dr. Taubenhaus to "present practically all the earmarks of the organism which I have described as *Sclerotium bataticola*." Examination in India showed that cultures of *S. bataticola*, obtained from America, were morphologically indistinguishable from cultures of M. *Corchori*, on the same medium, and infections were made with the former to see if the pycnidial stage of *M. Corchori* would result.

Infections with *S. bataticola*.

(1) Six jute plants growing in pots were wounded and infected with *S. bataticola* as in previous experiments. Two of the infected plants developed the disease and died, but pycnidia were not produced. Six control plants remained healthy.

(2) Thirty jute plants were wounded and infected in the field from cultures of *S. bataticola*. Twelve of these developed the symptoms of the disease, a

[1] Shaw, F. J. F. Morphology and Parasitism of *Rhizoctonia*. *Mem. Dept. Agri. India, Bot. Ser.*, Vol. IV, 1912.

[2] Taubenhaus, J. J. Black Rots of the Sweet Potato. *Phytopathology*, Vol. III, 1913.

[3] Martin, W. H. *Sclerotium bataticola*. *Phytopathology*, Vol. VII, 1917.

brown stain spreading from the seat of infection, but in no case were pycnidia produced.

From these experiments it appears that the fungus *S. bataticola* is a less virulent parasite of jute than *M. Corchori*, and that, up to the present, the American fungus has failed to give the pycnidium of *M. Corchori* when infected upon jute. If these two names both refer to the same organism we can only assume that the American form has lost the capacity of producing the pycnidium. In dealing with sclerotial fungi, however, it must be remembered that, when we only know these organisms in the vegetative stage, reliance for systematic purpose is placed on characters which would not be admitted of taxonomic importance were the reproductive organs known.

Summary.

(1) The fungus *Macrophoma Corchori* Syd. is the cause of stem rot of jute in India.

(2) The pycnidium occurs only on the jute plant.

(3) A sclerotial form occurs in culture and on other hosts (e.g., potato, cotton) in addition to jute.

(4) The amount of potash in the soil is a determining factor in the incidence of the disease.

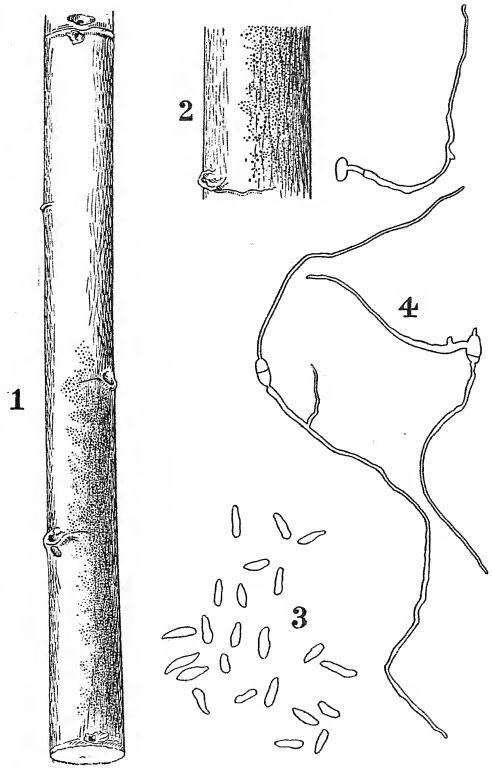

Fig. 1. 1, Stem of *C. capsularis* showing pycnidia of *M. Corchori*; 2, The same (× 4); 3, Spores, and 4, Germinating spores, of *M. Corchori* (× 194).

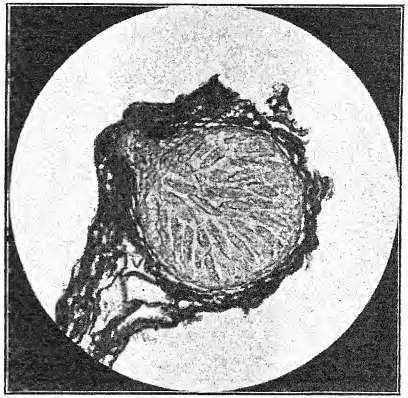

Fig. 2. Pycnidium of *M. Corchori*.

PLATE I.

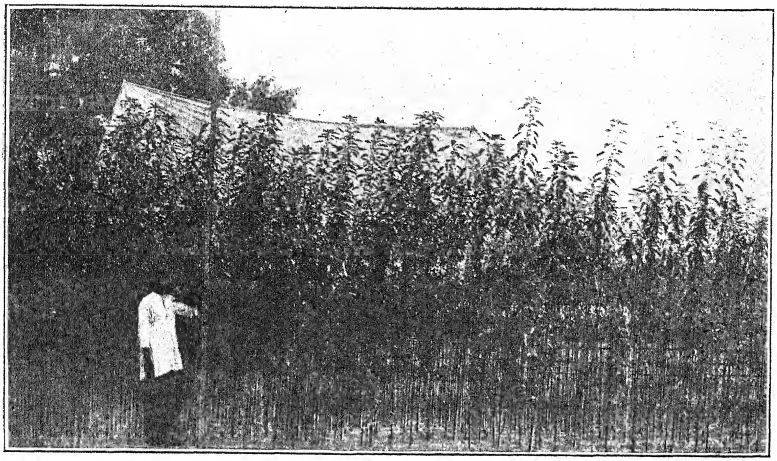

Fig. 1. A healthy plot of *C. capsularis*.

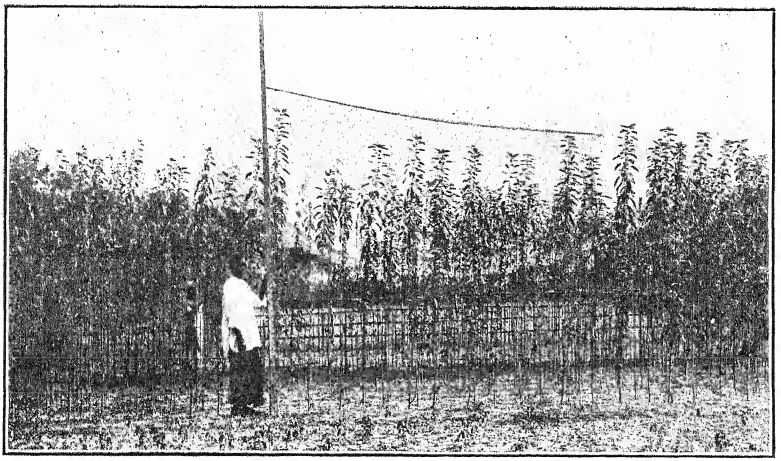

Fig. 2. Plot of *C. capsularis* with stem rot (*M. Corchori*) disease.